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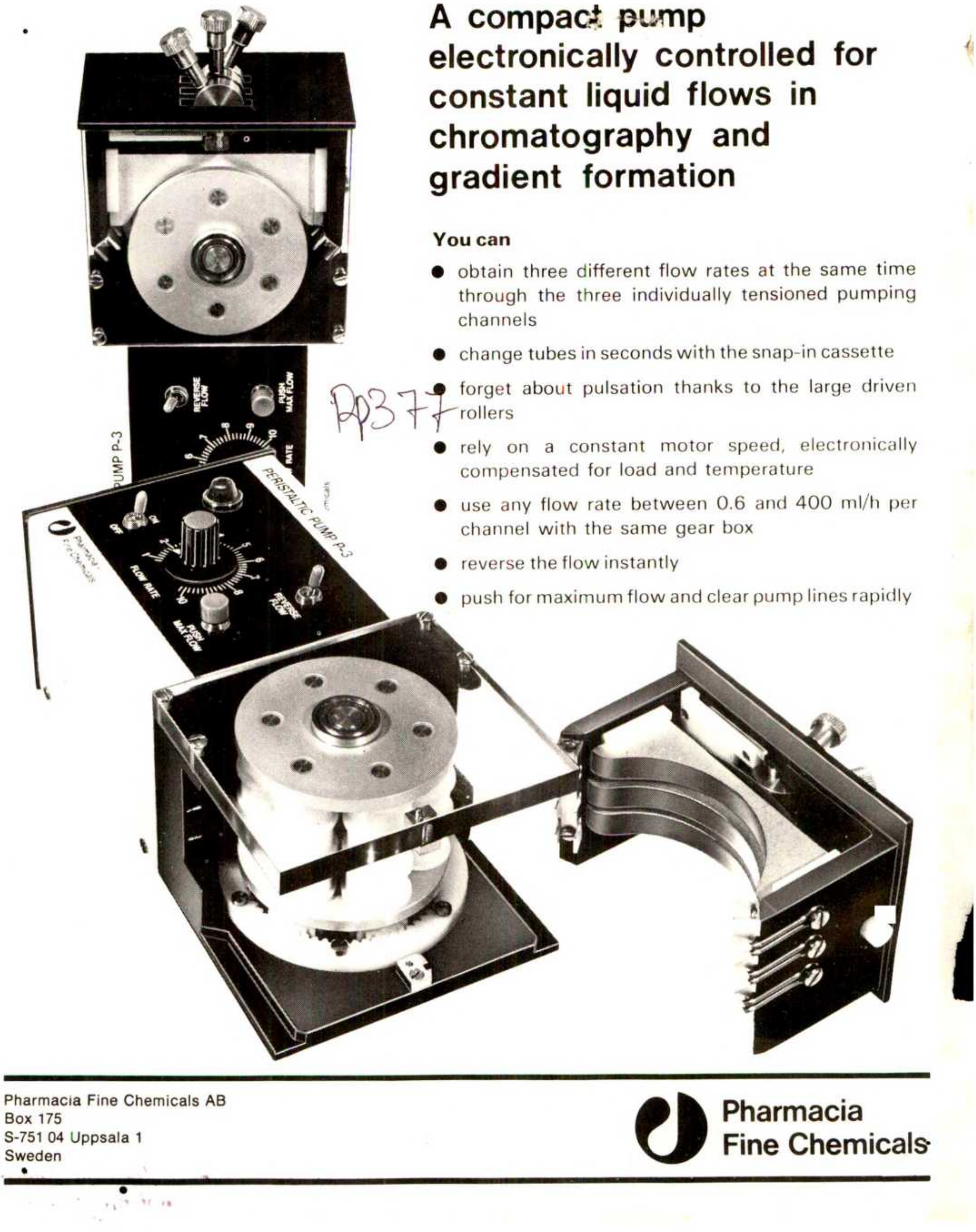

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A photograph for Boston's Museum of Science by Steve Cosmopalus See page 85 for a review of the museum

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A hundred years ago

We are glad to see that a contemporary not specially devoted to science—the *Morning Post*—in an article on Dr. Hooker's address at Belfast, points out to its readers that the majority of the observations referred to could be made "by any intelligent person without any scientific training," and expresses a hope that "people who have the opportunities for cultivating, and leisure for observing, will make collections of plants . . . . and add to our stock of knowledge." At the same time it suggested these as interesting subjects for observation :—" How much can plants eat in twenty-four hours? When do they eat most? Under what conditions of weather? &c. Indeed, the whole field is one that is almost unexplored." May this hint, which will reach many who are not readers of scientific papers, not be without result! We would draw attention to the fact that plants of *Drosera rotundifolia* are advertised for sale at ninepence each, and we hope that before long some enterprising dealer may make a speciality of all known carnivorous plants for suitable observations.

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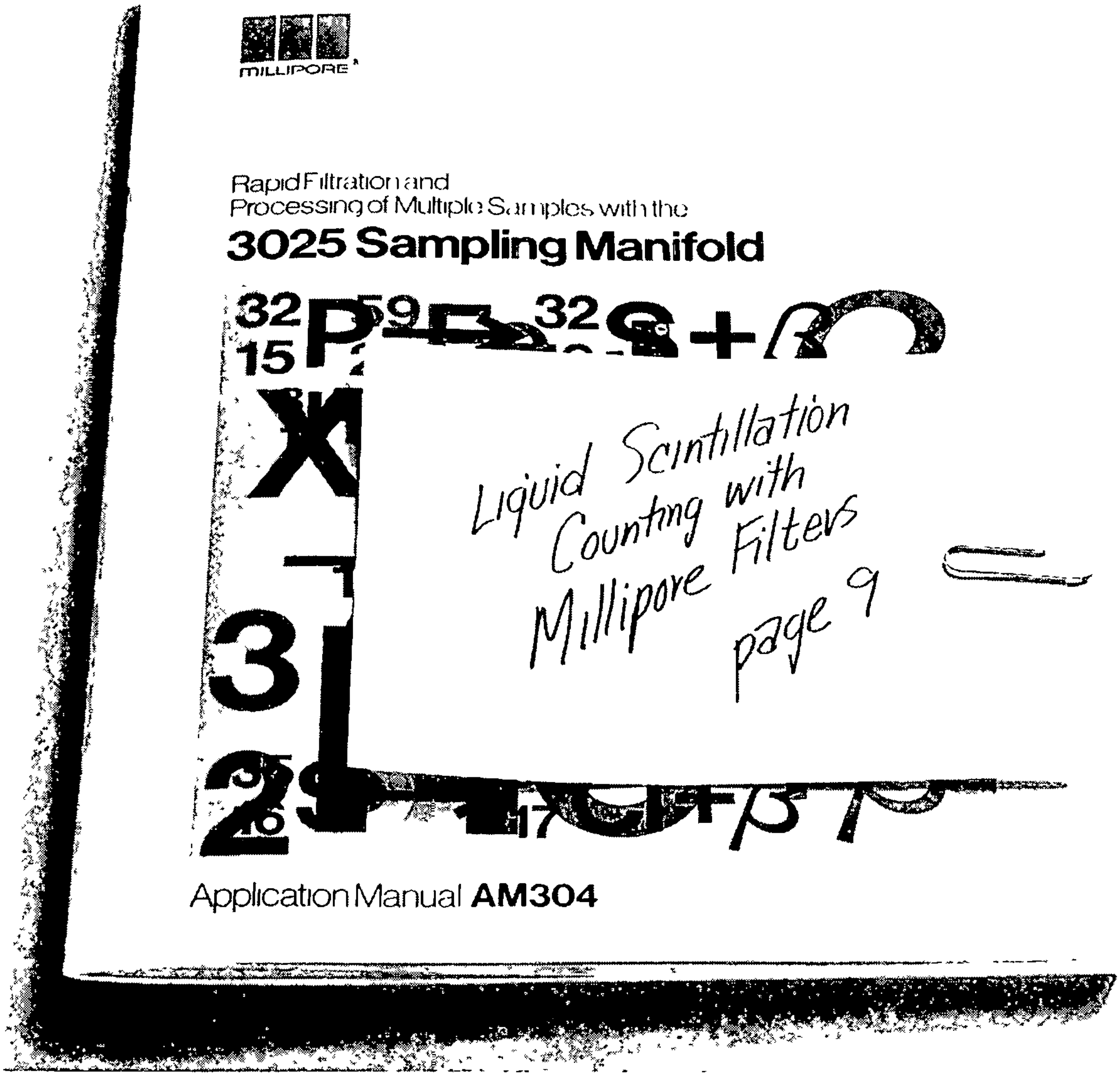

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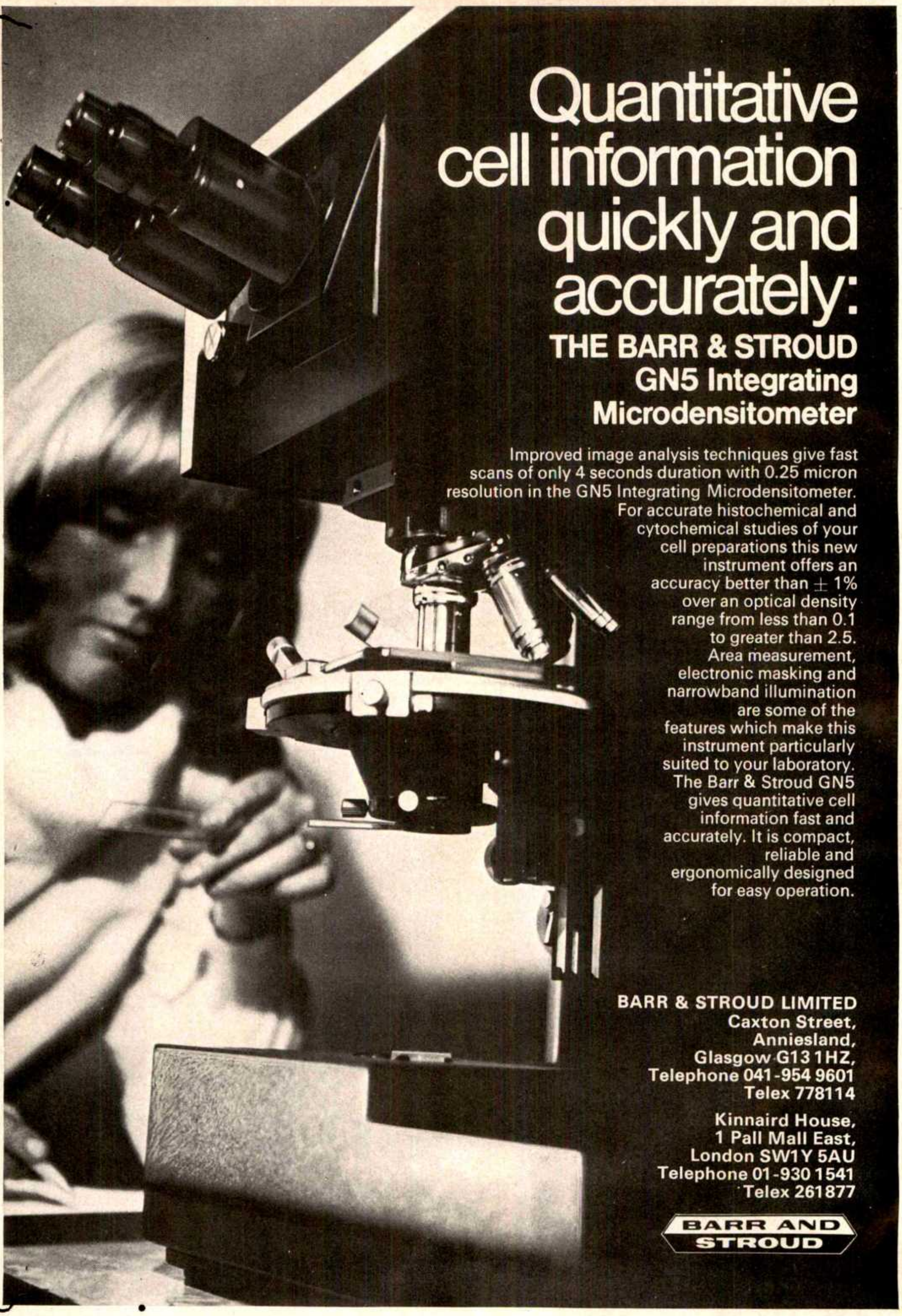

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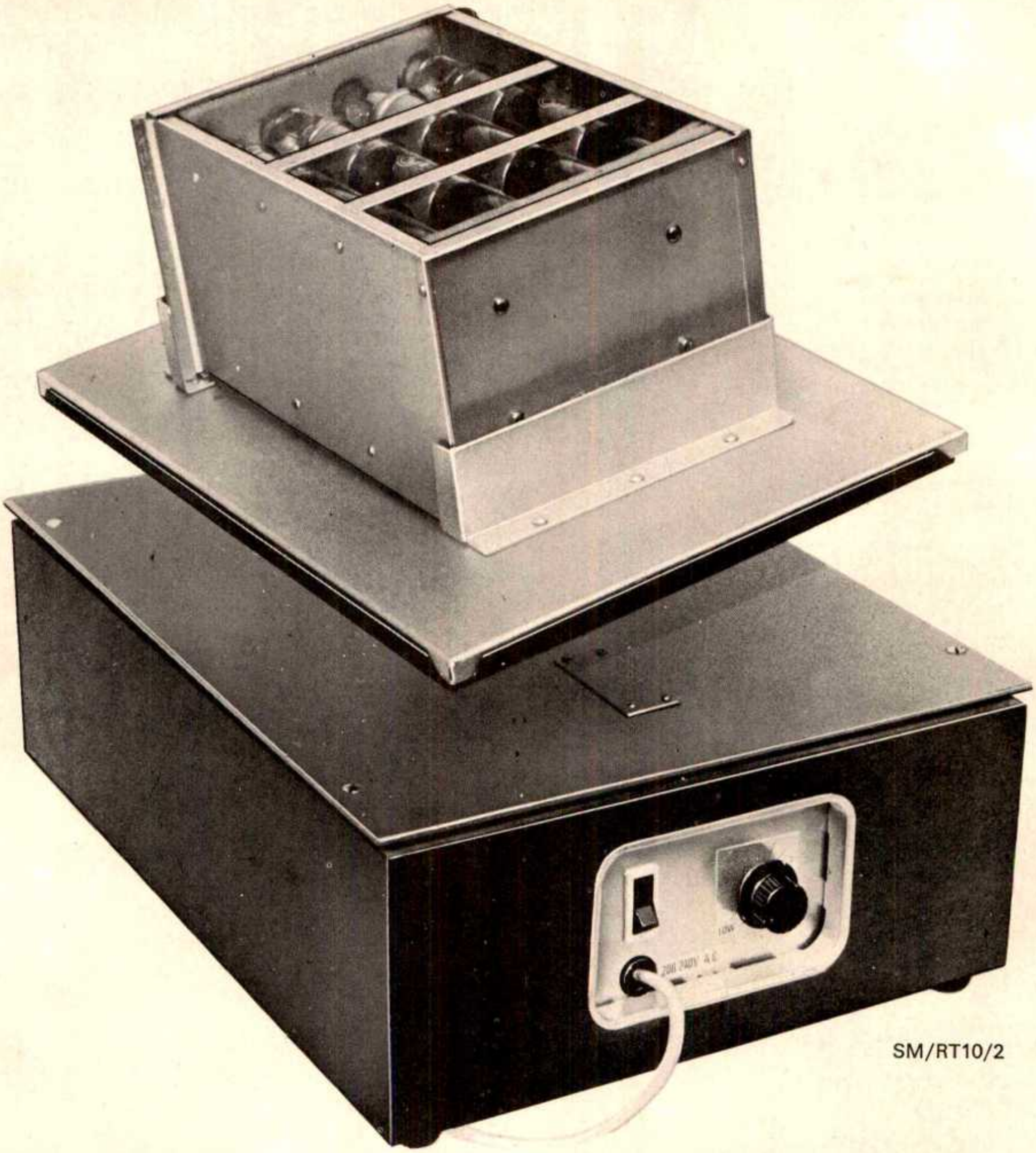

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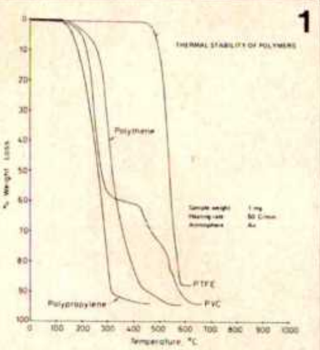
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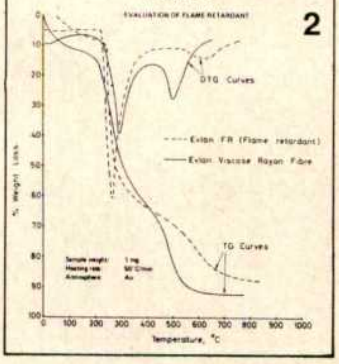
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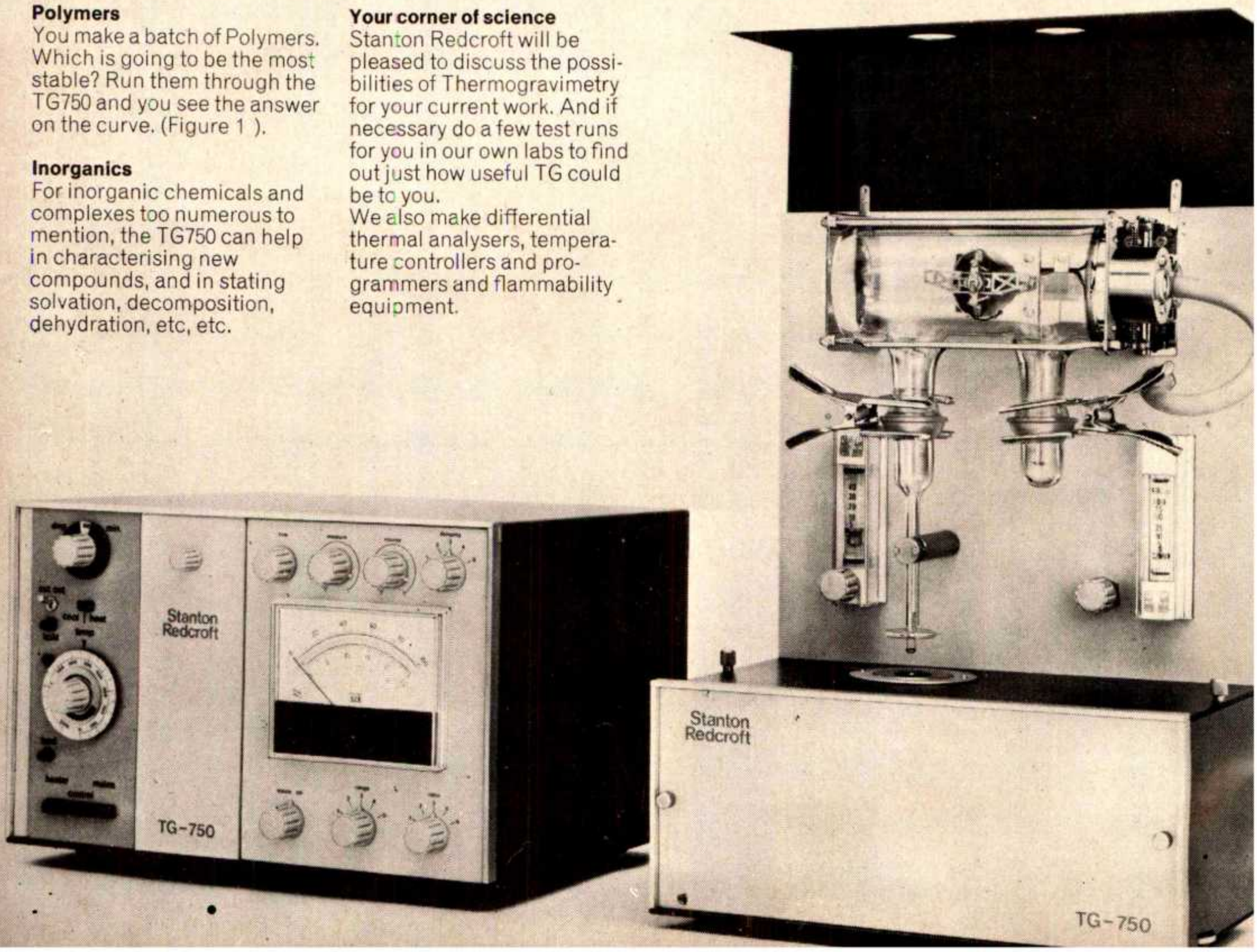


nature

Volume 251 September 6, 1974

Should we publicise those experiments?

THE vigorous debate, started several weeks ago by the appeal from a panel of the National Academy of Sciences for self-restraint in pursuing research on certain types of plasmid engineering, does not as yet show any sign of waning. True, the number of scientists technically qualified to appreciate the risks involved and weigh them against other considerations is relatively small, but each of them is going to wish to make a very personal contribution to the discussion since it probably concerns his way of going about research in the future. Further, there are many more scientists who may understand little or nothing of the technical content of the appeal but who can envisage quite well the sort of feelings that are aroused when a distinguished group of scientists proposes a halt to certain research work.

One of the ramifications of the proposed ban is that scientific journals might wish to take note of it in their publication policy. Thus David Baltimore, one of the NAS committee members, said "editorial boards . . . will probably think twice about publishing research papers derived from experiments covered by the embargo", and Michael Stoker, Director of the Imperial Cancer Research Fund, wrote "editors . . . will also need to consider their policies in the face of wide support for a moratorium".

We are obviously capable of speaking only for *Nature* but it is our opinion that any policy that tries to eliminate from the journal reports of experiments that are believed—even widely believed—to be potentially dangerous would be quite wrong. We are unsure that editors of more specialised journals would necessarily feel similarly.

There are two reasons why we feel that *Nature* should not take a stand against such papers. The first is that asking a referee to remark on the morality of a paper (assuming that it is not a question of legality) cannot but lead to impossible situations. Should reports of nuclear fission have been called 'potentially dangerous' and in bad taste? Should scientific work emerging from nuclear detonations or weather modification be kept out of the literature? Is the administering of electric shocks to animals cruel? These are not questions which can safely be left to a referee with his own personal predilections, or even an editor with his, and to attempt to do so by putting an X in the box if you believe the experiment should never have been done in the first place is rather like asking a judge to deliver his verdict only after he has factored in to his decisions his own feelings about whether the law was a good one to start with. The community can either legislate against certain experiments or it can attempt to persuade practitioners to desist; if it tries to put the onus on an individual to decide for the community, it can hardly be surprised if the result is often quirky.

The second reason why we would not automatically decline papers describing things of which many would disapprove is that it is surely more important that these things be given publicity than that they be pushed into the underground.

This hardly means that we would actively solicit such manuscripts nor that we would be more likely to accept them in order to take advantage of their obviously unusual character. But if a scientist has chosen to ignore a widespread call then a case could occasionally be made for drawing the community's attention to who he is and what he is doing. Besides, proscribed experiments will need continued re-evaluation in the light of new techniques and new understandings, and it may be that research done in forbidden territory, however lacking in approval by a majority of scientists, positively helps in reinforcing or easing the moratorium.

We have a broad general sympathy with the aims of the NAS group and we recognise in their statement important ground-breaking in the scientific community. Nevertheless, we would not at present wish to commit ourselves to any blanket policy of turning away reports from those who work on regardless. The issue of publication, though, certainly merits an airing at next February's international meeting.

A hundred years ago

While progress has been made with gigantic strides in many directions, in engineering and in mechanics generally; while railways, steamboats, and electric telegraphs have extended their wonders to the most distant parts of the world; and while trade, with these aids, is bringing to our shores the produce even of the most distant places, to add to our comforts and our luxuries; yet, when we come to look to our homes, to the places where most of our population have to spend nearly the whole of their lives, I think we must find, with regret, that, in matters pertaining to the salubrity and general amenities of our towns and houses, as places for residence, due progress in improvement has not been made. Our house drainage arrangements are habitually disgracefully bad; and this I proclaim emphatically, alike in reference to the houses of the rich and the poor. We have got, since the early part of the present century, the benefit of the light of gas in our apartments; but we allow the pernicious products of combustion to gather in large quantities in the air we have to breathe; and in winter evenings we live with our heads in heated and vitiated air, while our feet are ventilated with a current of fresh, cold air, gliding along the floor towards the fireplace to be drawn uselessly up the chimney. A very few people have commenced to provide chimneys or flues to carry away the fumes of their more important gaslights, in like manner as we have chimneys for our ordinary fires. In mentioning this, however, as a suggestion of the course in which improvement ought to advance, I feel bound to offer a few words of caution against the introduction of flue pipes for the gas flames rashly, in such ways as to bring danger of their setting fire to the house.

From the Presidential Address of Prof James Thompson to the Mechanical Science Section of the British Association, in *Nature*, **10**, 393, Sept 10, 1874.

Senator Kennedy: inspired anti-foetal research bill.

Guinea-pig charters

A number of ethically questionable research practices brought to light recently in the United States have led to calls for tighter control of human experimentation. Colin Norman discusses some of the regulations which have been proposed and their effect on research.

MANY American scientists hoping to conduct experiments on their fellow men and women will soon have to submit the ethics of their research to intense scrutiny by a raft of committees before they can get any funds. Some will then find people constantly looking over their shoulders while they carry out their experiments, others will find whole avenues of research shut off by the federal government or by state and local laws. In short, the traditional freedom of the scientist to organise his work is about to see drastic changes.

Human experimentation, like presidential power, has recently been a subject of national debate thanks to some widely publicised examples of ethical bankruptcy. Such examples include allegations that a number of poor black men who were known to have syphilis were left untreated so that the course of their disease could be monitored, that prisoners have been subjected to painful and essentially pointless medical experiments and that several children have been left mentally maimed by psychosurgery. The allegations have led to strident demands for more control over human experimentation, and the vocal anti-abortion lobby in the United States has also left its mark in the area of foetal research.

The controls have come from four directions: Congress, the Department of Health, Education and Welfare (HEW), state and local governments, and the courts.

At the Congressional level, a bill has been passed and signed into law by former President Nixon which sets up an eleven-member national commission on human experimentation whose duty will be to draft a set of regulations to protect human subjects of biomedical and behavioural research. The commission, which should be established later this month, has been given two years to complete the task and has been asked to concentrate its attention on areas such as research with children, prisoners and the mentally disabled, where ethical and legal problems abound.

As for research on pregnant women and foetuses, the bill—a measure inspired by Senator Edward Kennedy called the National Research Act—imposes a four-month ban on federal support for research involving foetuses with beating hearts, before or after an induced abortion. While the ban is in effect, the commission will draw up regulations governing such research.

Meanwhile, HEW has been moving independently to strengthen controls over human experimentation supported by the National Institute of Health (NIH), the National Institute of Mental Health (NIMH), the Food and Drug Administration (FDA) and other HEW agencies.

The department has already imposed a blanket provision that human experimentation will only be supported at institutions which have established review committees to scrutinise the ethics of individual studies before application is made for funds, and it is now in the middle of devising extra protection for children, pregnant women, foetuses, prisoners and mental patients. Last week, HEW proposed some tough regulations in that regard.

At the state and local level, several laws have been passed which impose restrictions on foetal research, the most prominent being a recent move in Massachusetts to outlaw research on living foetuses, before or after abortion. While the federal regulations would simply cut off funding for such research, the state laws would make some types of foetal research a crime punishable by a stretch in jail.

As for the courts, the whole concept of legal consent to take part in research as it applies to children and mental patients is under challenge in at least two cases, and in Boston four doctors are now awaiting trial on charges arising from some foetal research they conducted in 1971. Thus, as is common practice in the United States, the courts are likely to define some of the parameters of social policy.

All these attempts to regulate the practice of human experimentation involve a delicate balancing act by weighing potential benefits to society from medical research against the possible risks to those who take part. It is a balancing act which has its roots in the preamble to the United States Constitution, which states that the Constitution is designed to "promote the general Welfare, and to secure the Blessings of Liberty".

According to Dr Donald T. Chalkley, chief of NIH's Instutional Relations Branch, which has been drafting the HEW regulations, the real problem is that "we have a public concept of research that is based on fiction—on Clockwork Orange or Lord of the Flies experiments—but 90% of the research involves no more than the taking of a blood sample". This public concept is causing legislators to pass unduly restrictive laws, such as the blanket ban on foetal research.

Although stopping well short of imposing a flat ban on research involving living foetuses, HEW's proposed regulations would severely restrict such studies, and outlaw some kinds of research entirely. In short, such experiments would be subjected to three separate ethical checks, and they would have to be confined to a very narrow range of experiments in order to qualify for HEW support.

Ruled out would be a wide range of experiments on foetuses *in utero*, before they are aborted. Such prohibitions are also embodied in the Massachusetts foetal research law, and when that law was being debated, many scientists warned that the effect would be to stop a number of valuable research projects, and possibly to place future generations of children at risk.

The prohibition would prevent drugs or vaccines being administered to pregnant women about to undergo abortions in order to see whether they cross the placenta into the foetus For some substances, such as rubella vaccine, such knowledge is essential because of the dangers that foetal abnormalities may be caused if the substance does cross the placenta Dr Chalkley acknowledged in an interview last week that banning such research could place children at risk, but he pointed out that the problem is to balance the individual rights of the foetus and its mother against the potential promotion of the general welfare of children, both of which are equally protected by the Constitution

HEW also chose to draw the line against foetal research *in utero,* which is not designed to benefit the mother or that particular foetus, because if the foetus is placed at risk by such experiments, it would be very difficult for the mother to change her mind about going through with the abortion

It is worth pointing out, however, that although the HEW proposals would permit experiments designed to benefit the mother or the foetus, they would rule out control experiments because they ban all non-therapeutic studies on living foetuses

If the setting of regulations governing foetal research is fraught with difficulty, the situation regarding research on children is even more difficult The benefits from paediatric research are obvious, but the ethical and legal problems are also legion, and HEW, which has been trying to set some regulations in this area for the past couple of years has so far been unable to do so

The problem is essentially two-fold First, there is the difficulty of defining what kinds of research should be permitted and what should be outlawed As Chalkley put it last week, if you shut off an area of research now because the potential benefits are outweighed by the risks, you may be opening the door to new diseases And second, there is the problem of getting informed consent from minors

Can a parent or guardian legally and morally consent to have his or her child take part in a research project which might involve some risk, but which will not benefit the child directly? A law suit, filed against the University of California by an attorney, James R Nielsen, contends that no "invasive" medical research procedures should be practised on a normal healthy child unless they hold potential direct benefit for the child

Again, if research directly beneficial to the child is allowed but every other type of paediatric research is outlawed, one effect would be to prevent the use of control groups in any such experiment Clearly, the issue of human experimentation is far from dead, and verbal battles will be fought on a variety of fronts But at present the course of events at the federal level is far from clear

The most likely scenario, is that HEW (open for comments until November) will put its regulations into effect next year, and the commission will suggest amendments in 1976 ☐

LAST month, the US Department of Health, Education and Welfare (HEW) proposed some tough regulations governing research on prisoners, mental patients, pregnant women and foetuses Designed to protect the rights of those being experimented upon, the regulations would establish a number of ethical checks on research projects, and even ban some types of experiments entirely

First, under regulations already in effect, every institution in receipt of funds from HEW must establish an Organisational Review Committee, composed of people from a variety of occupations, to review research protocols involving human experimentation before they are submitted to HEW for funding This first ethical check will weigh the benefits to be derived from the experiment against the risks to those taking part, and it will also ensure that adequate provision is made for obtaining informed consent from the subjects

In addition, HEW is now proposing that another committee—the Consent Committee—be established at the institutional level to provide continuing ethical review of experiments involving prisoners, mental patients and pregnant women This committee will oversee the actual selection of subjects, make sure that they consent to take part without coercion and after having been fully informed of the risks, and that they are able to opt out at any stage if they no longer want to take part The new HEW proposals would also impose the following restrictions

- By far the most controversial portion of the proposed regulations deals with research on pregnant women and foetuses First, HEW is suggesting that yet another ethical check be applied to such studies from an Ethical Advisory Board, to be established in every HEW agency funding foetal research

These boards, which would consist of members appointed by the Secretary of HEW, from outside the government would review all grant applications for such studies after they have been approved by Organisational Review Committees

All research involving foetuses *in utero* on pregnant women would, however, be banned unless (a) "the purpose of the activity is to benefit the particular foetus or to respond to the health needs of the mother", or (b) the research is aimed at "evaluating or improving methods of prenatal diagnosis, methods of prevention of premature birth, or methods of intervention to offset the effects of genetic abnormality or cengenital injury", in which case, "the activity [must be] conducted as part of (but not prior to the commencement of) a procedure to terminate the pregnancy" The mother's consent would be required for such research, and the researcher should have no part in determining either the timing or method of abortion or the viability of the foetus

As for research on abortuses, the proposed regulations would require that relevant animal studies first be completed, that the mother's (and if possible the father's) consent be given, and that the vital functions of the abortus are not artificially maintained Furthermore, HEW is proposing that experimental procedures which would terminate the heartbeat or respiration of the foetus should be banned, but it stops short of calling for complete cessation of studies on foetuses with beating hearts

- As far as research on prisoners is concerned, HEW suggests that an Organisation Review Committee and a Consent Committee should provide adequate protection for research subjects, by making sure that prisoners are offered no special inducements or threats to make them take part A preamble to the regulations specifically rejects the notion that since prisoners have little freedom of choice in anything, they are incapable of freely giving their consent to take part in research projects
- Involuntarily detained mental patients pose a slightly more difficult problem The regulations, however, would permit research on such people provided that the study is "related to the etiology, pathogenesis, prevention, diagnosis, or treatment of mental disability or the management, training, or rehabilitation of the mentally disabled and seeks information which cannot be obtained from subjects who are not institutionalised mentally disabled"

In other words, HEW is attempting to rule out the use of mental patients for studies unrelated to their mental illness

The proposed regulations are available from the Institutional Relations Branch, Division of Research Grants, NIH, Bethesda, Md 20014, USA

international news

B.A.—first the bad news

OPENING this year's British Association conference at Stirling, the President, Sir John Kendrew, called for changing attitudes amongst scientists to counteract the growing indifference and even hostility towards science shown by the non-scientific public

A note of gloom pervaded the first few minutes of the address, as Sir John outlined the problems facing the practice of science today The relationship of science with society has become slightly defensive on the part of the scientists Increasing financial restraint on scientific research and the downgrading of scientific adviser posts both in Britain and the United States have not helped matters

The hostility towards science arose because people saw it as the handmaiden of economic growth, as the creator of pollution, as a support for the military and as a source of little understood dangers such as genetic engineering and computer data banks, or even as a negation of humanity itself

But, said Sir John, changing to a more optimistic note, the scientists' feelings of pessimism and guilt are unjustified Science has proved itself in the past and there will be an absolute need for science in the future But hostile critics will only be converted by a change in scientists' attitudes Scientists must focus on the challenges which will face us in the future and be less despondent about some of the immediate practical difficulties

The main challenge was to decrease the widening gap between our standards of living and those of the poorer peoples Whether we favour a "growth" or a 'no-growth" society said Sir John, we could not be content that a no-growth society should involve most of the world's population living in undernourishment, squalor and disease

The concept that the pursuit of knowledge as an absolute good in itself was the prime motive of scientific research needed to be re-examined, said Sir John Although a good motive, it had not been, nor ever could be the only one After the War, in which many scientists had applied their skills, there had been a reaction towards pure research for its own sake, he said But the world had changed since the 1950s and the challenges had changed, too "In my view," said Sir John, "it is neither socially productive nor intellectually satisfying to refuse to modify the old attitudes or to respond to the new challenges " Much contemporary research might seem to future historians like the minute and pettifogging scholasticism of mediaeval times

Even the increasingly tight research budgets might compel scientists to direct research into fields that may actually turn out to be more productive than those that could be afforded in times of plenty Limited budgets could also be optimised through international cooperation, despite the pitfalls of bureaucracy, politics and commercial restraint

Sir John went on to discuss specifically the problems arising from the genetic manipulation of bacteria, an example of a scientific advance which could hold dangers for the human race and which has recently been much debated in public

In these experiments. pieces from animals or viruses can be linked with bacterial DNA in such a way that the complete DNA molecule can replicate inside a bacterium It was feared that the deliberate or accidental introduction of cancer-causing genes into bacteria for further study—an admirable end in itself—could lead to these genes becoming widely disseminated in the human and animal population, since the organism most widely used for this type of work was the common gut bacterium, *Escherichia coli* But the incorporation of certain animal genes making useful products could be of great benefit No-one yet knew or could foresee, all the possible dangers as these techniques were still relatively untried

Sir John drew an analogy with the state of nuclear physics at the beginning of the last War, when it became clear that nuclear fission could make possible the development of new power sources but could also be used to construct an atomic bomb

He approved the action of some American scientists and the British Medical Research Council in calling for a voluntary moratorium until the dangers of this work had been evaluated A permanent international body made up of molecular biologists themselves, should recommend what types of experiments should or should not be carried out and under what conditions of security Also a fact-finding programme of research in this area should be conducted under conditions of high security and the results published fully in the open scientific literature

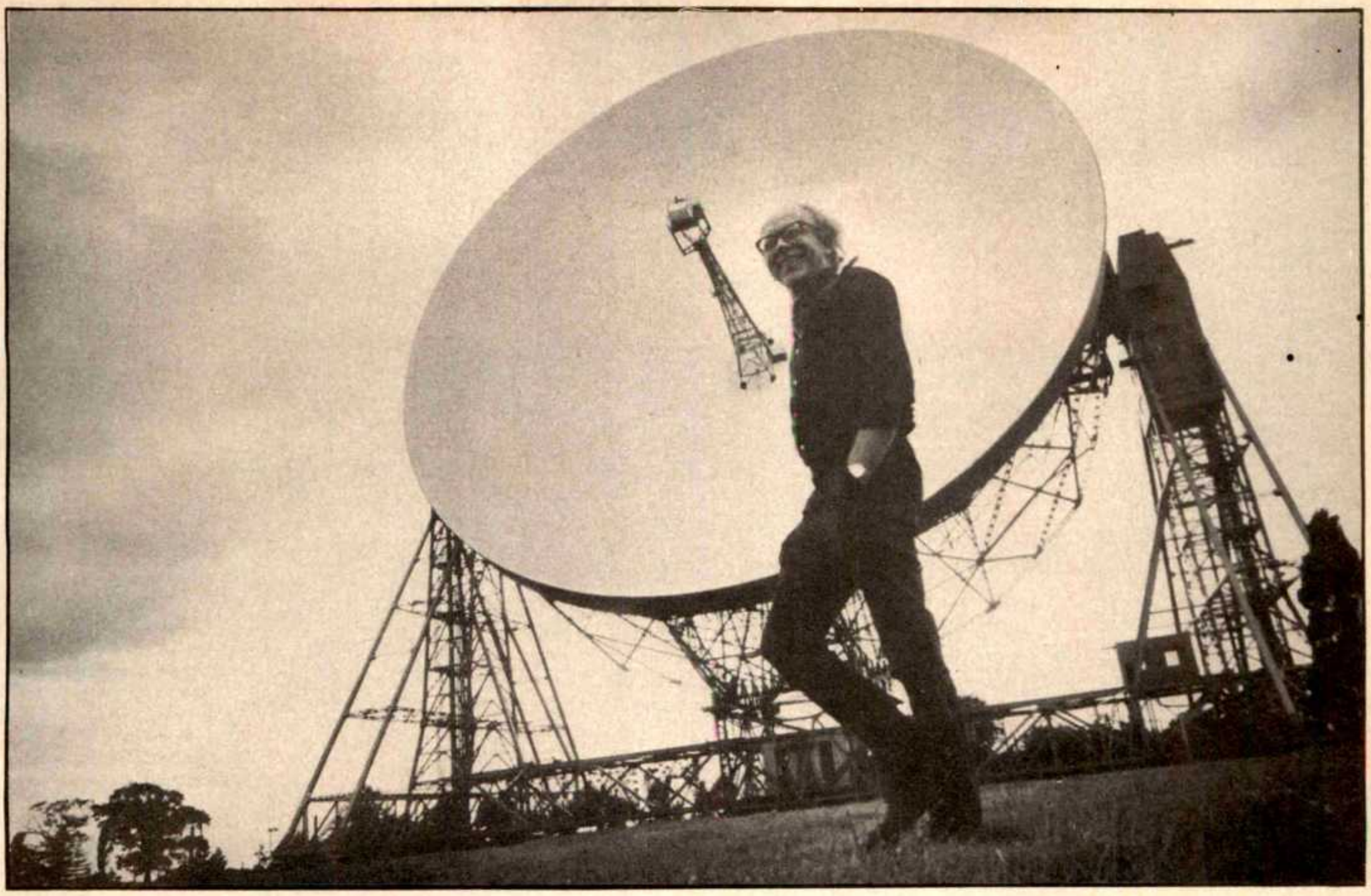
This was a case, he said, where scientists must be seen to put their own house in order, or else others would step in and do it for them perhaps in ways which would lead to quite undesirable restrictions on what they do

Even when decisions passed to the social or political forum, it was absolutely necessary that there should be effective communication of scientific knowledge upon which decisions could be based Communication of science to the general public seemed to be failing at present and scientists should take trouble to translate their ideas and discoveries into language which anyone could understand ☐

Science is fun

ON Saturday, August 31, Westfield College of the University of London held a carnival of science which it dubbed 'WES-POP' The idea for this feast of fun grew out of a similar but larger event held at Aix-en-Provence last year That event —AIX-POP—was arranged to coincide with a gathering of scientists from many nations for a conference on elementary particles In spite of some initial scepticism among the physicists cajoled into cooperating, AIX-POP proved a roaring success, in that hundreds of ordinary citizens were brought into contact with the scientists in small groups, where the scientists were forced to communicate intelligibly at a personal level

Professor Elliot Leader, of Westfield College, was one of the participants in the Aix event, and was sufficiently enthusiastic about the idea to organise something along the same lines in north-west London The event was widely publicised locally by means of posters in fairground style offering such treats as "See your friends bombarded by cosmic rays! " and "Watch the amazing lasers in action" And further publicity was lavishly provided by the local newspaper, which allowed Professor Leader space for three feature articles about physics and the festival—that in itself is remarkable enough for any British newspaper ☐



New director for the RGO

by John Gribbin

Professor F. Graham Smith, presently Professor of Radio Astronomy at the University of Manchester, is to succeed Dr Alan Hunter as Director of the Royal Greenwich Observatory (RGO) at Herstmonceux, Sussex. Stated that baldly, the appointment might cause consternation—what could be in the minds of the members of the Science Research Council (SRC) that they should appoint a radio astronomer to head England's principal optical observatory? But this superficially bizarre decision will not seem so odd to anyone who has followed the recent development of astronomy in Britain, nor to anyone who is familiar with Professor Smith's career; indeed, this could be one of the best things to happen to optical astronomy in Britain for a long time.

The situation has been confused, of course, by Professor Margaret Burbidge's brief yet stormy reign as Director of the RGO. On her resignation the then Deputy Director, Dr Hunter, took over the top job. But that could only be an interim appointment, since Dr Hunter reaches retiring age at the end of 1975, after 38 years with the observatory.

Now that situation is resolved. Professor Smith (pictured above at Jodrell Bank) will join the RGO staff this autumn, and will serve as deputy to Dr Hunter until the end of 1975. This should be ample time for him to learn the ropes, if past experience is anything to go by.

Professor Smith is far from being a narrow specialist in radio astronomical matters. Now 41, he completed the bulk of his formal education before radio astronomy developed as a discipline in its own right, and his PhD (awarded in 1952) must have been one of the very first awarded by the University of Cambridge for radio astronomy work. Following spells at the Telecommunications Research Establishment, Malvern, and at the Cavendish, he moved to his present post in Manchester in 1964. There can be no doubt that his influence is in no small measure responsible for the fact that Manchester is now recognised as a place where first class theoretical work in the field is done, as well as being the home of one of the world's leading radio astronomy observatories. In particular, Professor Smith has recently worked on theories of the emission mechanism of pulsars, cosmic rays and galactic radio sources.

In 1969, Professor Smith gained something of a reputation as a troubleshooter for the SRC, when he spent 10 months as Deputy Director of the Radio and Space Research Station (now the Appleton Laboratory) to assist with a review of the station's research policy. So Professor Smith takes with him to Herstmonceux a thorough knowledge of the workings of the SRC machinery and a great deal of administrative experience. There seems no likelihood that this appointment will fail either through personal conflicts between the Director and the SRC or through any lack of ability, and the RGO can thus look forward to a prolonged period of stable development. It is too early yet to see what direction that development will take, although the fact of stability alone will be welcome enough after recent troubles. But Professor Smith told *Nature* that he was particularly anxious to move to the RGO at once, rather than wait until 1975, in order to be fully involved with developing the proposed Northern Hemisphere Observatory. He emphasised that this is "a marvellous opportunity" to get to grips with the engineering side of optical astronomy, and to meet the people involved on a working basis, before he disappears into an office labelled 'Director'.

After much prevarication, that Northern Hemisphere Observatory now at last looks like becoming a reality. With Professor Smith playing an important role, and with the SRC's infrared telescope now definitely intended for a Hawaiian home, it begins to look as if there really will be a Northern Hemisphere Observatory, almost certainly in Hawaii, in the not too distant future. □

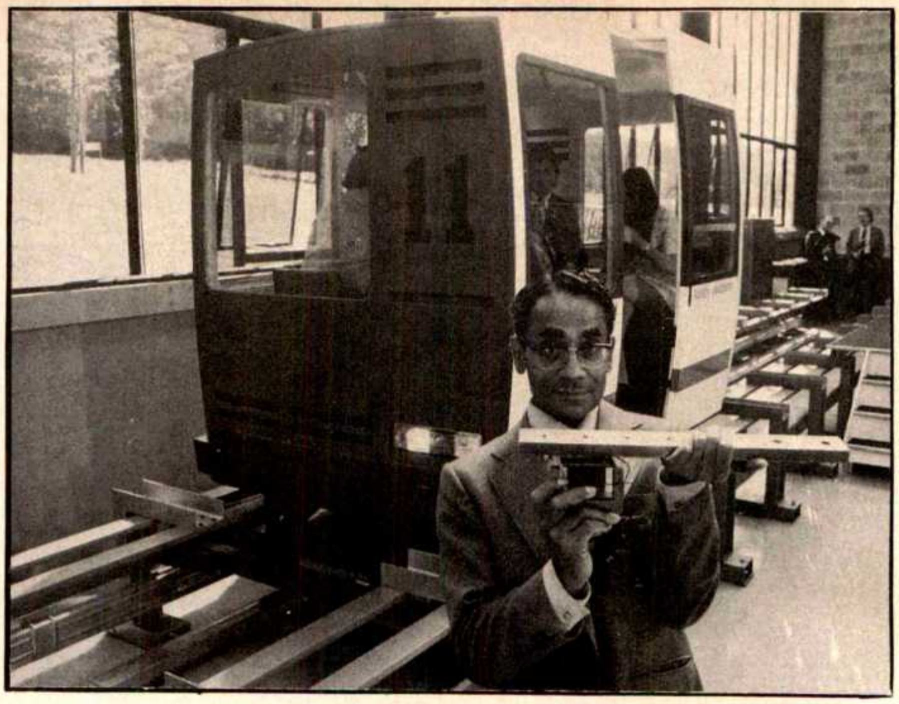
The case of the cancelled hovertrain

by Angela Croome

THE case of the British Government-funded experimental development of the hovertrain on which a White Paper (Tracked Hovercraft Ltd, Cmnd 5711) has just been issued is a classical example of how not to manage an innovative high-technology assessment project. What is so especially discouraging is that the sequence to cancellation could be predicted five years—and £5¼ million—earlier with very considerable reliability. More must have been written and minuted in Britain on the subject of fostering and managing technological innovation than almost any other technological subject since the last World War. Yet no lessons seem to have been learnt; no guidelines derived.

The White Paper has been produced under pressure from Parliament's Select Committee on Science and Technology which held an inquiry which took up much of the first half of 1973 and some 500 pages of reports, into the circumstances surrounding the close-down of Tracked Hovercraft Ltd. and its full-scale vehicle test track near Cambridge. The summary Report (July 24, 1973) of the Select Committee was very critical of almost every aspect of the business—of the cancellation of the project no less than the manner in which it was done (the bulk of the 150 staff were given less than 24 hours to be out of their offices, for example); of the Government's role, of the way innovation is managed (or mismanaged) in Britain and the adequacy of the National Research Development Corporation for this task. It specifically proposed that there should be a 'high-level' review of the organisation needed to develop innovation and asked that the Government look again at the 1972 Select Committee recommendation of a small expert Department headed by a Cabinet Minister to look after Government sponsored R and D; further, that the THL test track at Earith should not be scrapped but become a multiuser research facility run by a consortium of British universities with interests in novel surface transport and headed by Imperial College.

The White Paper in response to this swingeing assault can only be described as a mouse. It dodges out of dealing with the main criticisms by attributing them to a previous Government for which it was not responsible. Apart from enunciating the principle that "no promising technology ought to be stifled in infancy for lack of funds" it expresses entire satisfaction "that departmental organisations, supplemented by the NRDC, provide adequate machinery for Government participation in the development and exploitation of inventions". As to providing some funds for maintaining the Earith test track as a multi-user facility, it denies the need for such a centre and by withdrawing restrictions on dismantling it laid down last year invites the speedy scrapping of the track. In the overall field of high speed public transport "the Government considers that the present effort and expense are appropriate in scale and pace".



Professor Bhalchandra Jayavant demonstrates the principle of the Sussex magnetic train: all right for Brighton front, but how good at speed?

The present effort and expense in this field amount to this. There is the multi-million pound British Rail development of the Advanced Passenger Train (APT) envisaged as running at up to 250 mph and coming into service on existing rail tracks in the 1980s. The development of the linear induction motor for fast tracked vehicle propulsions which THL at Cambridge had been fostering through its links with Professor Eric Laithwaite at Imperial College has been passed to the Brush subsidiary of Hawker Siddeley with a £400,000 Government grant against an investment of £100,000 by the company which it has been remarked has never made a linear motor before. The Wolfson Foundation has put about a quarter of a million into research into mass transport vehicles employing magnetic propulsion and/or levitation, notably at Sussex and Warwick universities. Now the Science Research Council is putting up some funds for similar work in the universities. Two things may be said about this pattern of effort. None of the work—save possibly some of that in the universities—looks beyond 1985, the date which in his much-criticised evidence to the Select Committee the previous Transport Minister (Mr Peyton) considered his departmental limit. Secondly, coordination of these activities now involves three ministries and the public sector. The dangers of fragmentation of effort have been reiterated continually during the debate on the organisation of government R and D no less than over the Tracked Hovercraft inquiry. If communication and coordination was inadequate when only British Rail's APT project and NRDC's THL were involved as the Select Committee indicated, how much worse is it going to be now?

A glance at the scale and pace of effort in other countries provides some sharp contrasts. Japan has perhaps the largest programme in high speed mass transport development. It is costed in thousands of millions, very long-term, much interested in electro-magnetic levitation employing cryogenics and no less than 60 professors are said to be involved full time. The United States has in August taken the opposite step taken by the British Government last year by dropping further work on its high speed passenger train (called the Improved Passenger Train Project) and switching the $5.6 million involved into support of tracked hovercraft transport. A huge test site is under construction in the Colorado desert where five different types of trackway amounting to some 70 miles altogether are being laid out as the High Speed Ground Test Center to test types of high speed propulsion including linear motors and suspension including both air cushion and mag-lev (magnetic levitation); as well as alternative modes

Biomedical advances give rise to concern

Topics of public concern arising from recent medical advances are given an airing in the British Association's most recent publication, *Social Concern and Biological Advances* (British Association, 50p).

A wide-ranging committee of scientists, lawyers, Members of Parliament, theologians and others was set up in 1972 to investigate the social, ethical and legal implications of certain medical advances, notably those in the fields of *in vitro* fertilisation and genetic manipulation.

Discussing artificial insemination by donor (AID) the committee consider that the legal status of any child conceived through AID needs to be properly defined as such children now occupy a twilight sub-legal zone. The genetic makeup of the donor is another important issue, and the committee call for a decision as to whether prospective sperm donors should be examined medically and as far as it is possible, genetically. The control of sperm storage banks is another problem of some concern. The government should decide immediately whether private banks are to be allowed and standards and supervision for such banks should be defined.

The committee recommends that research on *in vitro* fertilisation should continue as its possible advantages outweigh potential disadvantages. But there is the potential for abuse, and so the medical profession and government should keep a careful eye on new developments in this field and should be prepared to act quickly to prevent abuse.

Genetic screening for some genetically determined crippling diseases is now relatively common. Although this is superficially of great potential for reducing the numbers of children born with severe mental and physical abnormalities, the moral problems at both the social and personal levels can cause acute distress.

Social attitudes towards abortion can influence both the doctor's recommendation and the mother's response when an abnormality it detected especially in borderline cases where the degree of the abnormality cannot be predicted precisely. Less obvious but equally distressing, is the risk that a woman carrying an affected foetus could be subjected to social censure if she refused to be screened or undergo an abortion. This censure could also carry over to the child itself.

Under the broad heading of genetic engineering, eugenics and pre-natal sexing, the committee considers gene therapy, potentially useful but still experimental; sterilisation of people carrying defective genes; and the possibility of being able to choose the sex of one's child.

A policy of selective sterilisation needs to be scrutinised particularly carefully, as individual rights must be protected and the definition of 'normality' can be open to abuse by political and social systems which seek to manipulate it for their own ends. The committee make no specific recommendations on this head but advise continuing review of developments in this field.

The committee seem undecided as to whether pre-natal sexing will have much appeal for Western societies but recommend that official bodies should be prepared to act quickly to prevent precipitous and unadvised applications of new sex selection procedures.

The report also covers the chief moral problems involved in organ transplantation such as defining the moment of death and the ownership of the dead body. "Any definition of death" it says, "implies an understanding of what constitutes life".

The 1961 Human Tissues Act covers the transplantation of organs but is unsatisfactory on some points. Under this Act the legal possessor of the dead body can authorise the removal of the organs. But who, asks the committee, is the legal possessor, and what constitutes a reasonable inquiry to gain permission from relatives to remove organs for transplantation? Also, should Britain operate a "contracting in" or a "contracting out" system whereby people indicate whether or not they wish their organs to be used after death?

The committee favour adopting some of the recommendations of the 1969 McLennan Committee. These are that the present system should stand but that it needs legal reinterpretation to remove ambiguities and to facilitate the supply of organs to transplantation. Registers should be kept both of those who wish to contract out, and on the other side of the coin, those who specifically wish to donate organs after their death should also be registered.

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of urban transport.

In Germany 45 miles of test track are now planned at a site near Augsburg and very large public sums (£100m for R and D alone) have been invested in high speed transport for the future with particular emphasis going to linear motor development and magnetic levitation. Krauss Maffei which won the contract for the Toronto project at about the period that the Government announced the cancellation of the tracked hovecraft has switched its design from the 'conventional' double-sided linear motor to the single-sided type first conceived by Professor Laithwaite and 'proved' on the THL vehicle which Krauss Maffei had visited.

In the light of this amount of world activity and interest the ostensible reason for last year's Government cancellation of the tracked hovercraft, that there was no customer or market for it, seems extraordinarily thin. In fact some of the by-products of THL—the single-sided linear induction motor, the box-beam track for instance—have, as described, been adopted in overseas projects. The Select Committee accepted that Britain had about a five-year lead in overall hovertrain technology—and this was being reflected in consultancy fees particularly in North America. Professor Laithwaite's even more recent breakthrough which he calls the 'magnetic river' and which offers propulsion and controlled suspension in three axes from the same transverse-flux linear motor running on normal mains AC seems to put British research in the field of high speed transport even further ahead. Professor Laithwaite claims that he will have a production prototype of this double-purpose motor within six months, and it is particularly suitable for speeds of over 250 mph (where British Rail's APT runs out). Similar claims for the University of Sussex electro-magnetic attraction system, recently demonstrated in an urban passenger carrying version being devoloped for a 2½-mile shuttle along Brighton front, seem likely to be misplaced. Over 50 mph, a serious drag, develops from eddy-currents set up in the track (a problem that Krauss Maffei are encountering in the Toronto project now). One is forced to believe that the only customers that the British Government's chief scientist departments accept are home customers and that in the case of the tracked hovercraft it was especially unfortunate that the customer department DoE was also the sponsoring department of British Rail and its pet the APT. Customers beyond 1985 are not being considered and their seems no mechanism for considering the general public as a customer.

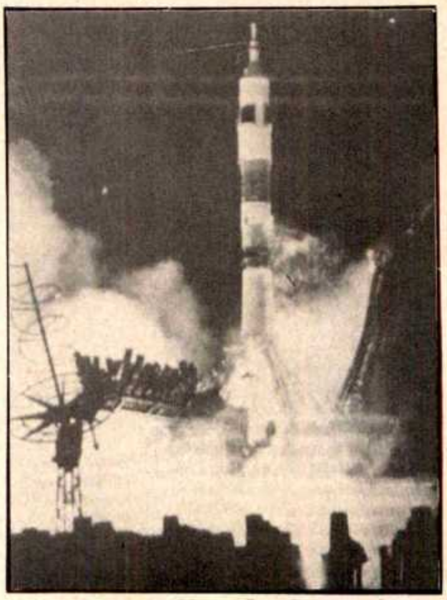
The Government has turned down the Imperial College form of proposal for keeping the Earith test track in being. Several practical problems now arise. The estimated cost of dismantling the track is £200,000 and THL has no funds left so the Government will

Soyuz – 15: didn't fall, wasn't pushed

from Vera Rich

THE brief flight of Soyuz-15 (August 26-28) has led to considerable speculation as to some possible failure which may have led to its curtailment. The report of a naked-eye sighting of Soyuz-15, which at 4.30 a.m. (GMT) on August 28 was seen to have overtaken the orbiting space-station Salyut-3, suggested that something had gone amiss, preventing an intended docking of the two craft.

In fact, however, the official *Tass* reports do little to substantiate these speculations. Although the first statement does mention Salyut-3 it does so only in an oblique manner, stating that: "The purpose of the launch of Soyuz-15 is the continuation of the scientific research and experiments in outer space begun on July 3 of this year in the flight of the transport ship Soyuz-14 and the station Salyut-3". The mention of Salyut-3 certainly suggested that the two craft would, in some way, participate in a joint experiment; the next *Tass* release, however, stated clearly that this would not be a link-up. The experiements were intended "to work out techniques of piloting the craft in different regimes of flight. In the process of manoeuvering the craft, Soyuz-15 made a number of approaches to the Salyut-3 station. The cosmonauts controlled the operation of all systems of the ship, carrying out observations of the stages of approach to the station". Soyuz-15, the release continued, was now preparing to return to earth.


Soyuz-15 blast-off: AP wire picture.

The *Tass* account of the descent stressed the details of the flight procedure—the use of the braking motors to align the craft before descent, the release of the parachute system and the firing of the braking motors immediately before touchdown. The aim of this last stage of the experiment was to test "methods and means of finding and evacuating a spaceship which had landed in night conditions". This was the first true night landing, although Soyuz-10 landed in predawn conditions. The search and recovery stage of Soviet space ventures has always held its own special hazards—one early pair of cosmonauts had to fight their way back to civilisation through the snow for two days Nevertheless, according to V. A. Shatanov, the commander of Soyuz-10, the need to practise night-landing is becoming more and more necessary, since the necessity to land at a given time of day imposes rigid conditions on the flight-plan even before take-off.

Tass reports are traditionally brief and uncommunicative, frustrating the desire for further information with such phrases as "according to its assigned programme", or "carried out its aims", without specifying full details of that programme or those aims. They are in a style therefore admirably suited to effect a cover-up of a partially aborted programme. But what if the speculations are wrong, and there was no break-down, and Soyuz-15 was planned from the beginning as a two-day mission. The need to practise night-landing, thereby eliminating rigidity of the flight plan, fits in well with the plans for the joint Soyuz-Apollo programme planned for 1975. (There could be considerable loss of face if either party had to ask for a revision of the joint flight-plan because its crew and ground support could not manage a landing under such-and-such conditions!) The practice of docking approaches from various positions, and the insistence that the cosmonauts were in total control of the craft is also interesting. There has always been a tendency in the Soviet manned space programme to incorporate a certain amount of ground control — the early cosmonauts were little more than passengers in the craft that carried them.

Speculation has suggested some technical failure in the Soyuz-15 craft, with possible setbacks to the Soyuz-Apollo programme. This may be the true explanation—but there remains the possibility that he *Tass* statements, do give, albeit briefly, the real objectives of the mission; that the flight of Soyuz-15 was intended simply to test out tricky manoeuvres, so that, in the joint mission, Soviet prestige might be duly maintained, by Soviet cosmonauts able to perform their part of the mission even in adverse circumstances—making docking approaches from any position, and able to cope with a touchdown at any hour of the day or night.

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have to find some kind of subvention to enable the company to remove the one mile existing track. There are two further miles of track in the form of unassembled beams in store at the manufacturers. What is to be done with them? With THL in nominal existence only, how are the numerous patents and the accumulated expertise to be exploited? It is impossible that the last has been heard of the tracked hovercraft and its company. Professor Laithwaite and his magnetic river are probably its greatest 'liquid' asset. He has not been heard from since the White Paper's publication but it is understood that alternative support is under discussion for the track and the plan to make it part of a possible joint European rapid transit centre.

There is a meeting of the EEC intercity working party in October in which ex-THL staff take part. The Select Committee may be regretting the move which enabled the Government to publish its meagre response while Parliament was in recess. This does not reflect on the excellence and thoroughness of the Committee's original Report and inquiry and there must be every expectation that it will either call for more evidence or make its views known through a debate on the White Paper as soon as Parliament is reconvened possibly accompanied by a further publication.

As to the sorry tale of the hovertrain's relevance for the bigger issue of the proper support of innovation today, Sir Christopher Cockerell, inventor of the parent concept, the hovercraft, gives the best summary (in a Memorandum to the Select Committee):

"To carry through a major development, a powerful lobby is required — example, Concorde; or a very powerful position — example British Rail and the high speed train; or prestige and many jobs at stake—example, Rolls Royce and the RB 211. . . . Now consider a brand new idea. It starts with neither money, facilities, a proven performance, a proven market, a lobby, prestige, jobs at stake, nor even people who understand it. . . . What is missing for big projects is a suitable machinery to cover the stages between NRDC's limited support and the fully commercial stage". □

news and views

Development of hominid locomotion

THE uniqueness of the human locomotor pattern has fascinated students of evolution for a long time and the past few years have seen an important increase in the complexity, detail and scope of locomotor studies Erect bipedalism is, of course, not found solely in man, but it is a rare form of locomotion in the animal world and both the reasons for its development and the nature of that development itself have stimulated much research

Although there is no consensus regarding why erect bipedalism has characterised the human lineage for at least several millions of years, there seems to be some agreement that no single factor 'caused' bipedality In other words, a combination of environmental and behavioural situations, combined with genetic changes, probably worked together in a positive feedback relationship For example, changes in the environment and behaviour might have provided new selective backgrounds favouring the incorporation of genetic advances in manual flexibility and dexterity, cerebral complexity and bipedality Changes of such physiological and anatomical nature could, in turn, provide new bases for further behavioural developments In this way, the development of bipedality is not viewed as an isolated event but rather as just one facet of highly interrelated and even interdependent evolutionary processes This approach to mutual causality in processes of social, cultural and physical development has come to be called "the second cybernetics" (Maruyama, *Am Sci* **51**, 164–179, 1963) Such reasoning is an important advance over earlier chicken-egg hypotheses in that it delineates the evolutionary process as taking place on several levels simultaneously A logical extension of this point of view is to suggest that it is the interrelationships between the levels which are in fact evolving and not the levels themselves

Most of the recent studies on human locomotion, however, have been more concerned with analysis of its physical development and biochemical characteristics Many different types of investigations have been used, anatomy, biomechanics, radiography, cineradiography, electromyography, as well as multivariate statistical techniques, have all been focused on the evaluation of locomotor patterns in living primates (including man) and some of this work has been used to reconstruct locomotor patterns in fossil groups (see Oxnard, *Syst Zool* **22**, 409–424, 1973 for a recent review) One result of the palaeontological investigations has been that despite the rarity of erect bipedalism in the animal world probably two slightly different forms of this locomotor pattern have been present, apparently simultaneously, within the human lineage The two patterns are contemporary at Lake Rudolf possibly as early as 3 million years ago and this suggests a profound and early dichotomy within the Hominidae Good evidence of this separation exists in the skull and dentition (Leakey, *Nature*, **237**, 264, 1972, Day and Leakey, *Am J phys Anthrop*, **39**, 341–354, 1973) and it is also demonstrated in the postcranium, most clearly in the femur, talus and tibia

In the human fossil record the femur is the best represented bone of the postcranial skeleton and in the relatively large number of femora now available two distinct patterns seem to have emerged One of these, the 'australopithecine' pattern, shows a small femoral head with a long flattened neck, non-flaring greater trochanter and a high degree of shaft obliquity or tilt (Walker, *J hum Evol* **2**, 545–555, 1973) Material showing this pattern is known from South Africa (SK82 and 97), Olduvai Gorge (OH20) (Day, *Symp Zool Soc London*, No 33, 29, 1973) and East Rudolf (KNM-ER 738, 815, 736 and 1505, Walker *op cit*) Moreover, radiographic evidence of the internal structure shows some possible distinctions in a greater thickness of the shaft walls and in the orientation and distribution of the internal trabecular structures (Walker, *ibid*) Within the Lake Rudolf material a second morphological complex has been identified and this pattern conforms more closely with that seen in living man a larger femoral head, shorter and rounder neck and flaring greater trochanter are the most visible similarities KNM-ER 737 (Day and Leakey, *Am J phys Anthrop*, **39**, 341, 1973) and KNM-ER 1481 and 1473 (Leakey, *Nature*, **242**, 447, 1973) show this pattern and have, in fact, been attributed to the genus *Homo* Further evidence of the dichotomy may also exist within the talus The talus is an important bone for locomotor assessment since it acts as a hinge joint between the lower leg and the foot and in this position can potentially reveal much about the structure and function of the foot Though the talar specimens TM 1517 (from Kromdraai), Olduvai Hominid 8, and KNM-ER 1476 and 1500, show some interesting variations within the group they seem to form one morphological pattern TM 1517 and OH 8 have been intensively evaluated through anatomical and multivariate techniques (Day and Wood, *Man*, **3**, 440, 1968) and through multivariate techniques alone (Oxnard, 1973), OH 8 has been subjected additionally, to biomechanical analysis (Preuschoft, *Folia Primatal* **14**, 209–240, 1971) Although there is agreement that the two specimens share many features they may have differed somewhat in the orientation and functional capabilities of the great toe The suggestion of the Kromdraai talus is that the great toe was somewhat divergent (Le Gros Clark, *J Anat Lond* **81**, 300, 1947) and this may have resulted in a more flexible foot capable of "some climbing ability" (Robinson, *Early Hominid Posture and Locomotion*, University of Chicago Press, 1972) and some "grasping" ability (Le Gros Clark, *op cit*) The OH 8 specimen (a nearly complete foot), has, however, a great toe which is not significantly divergent although Preuschoft (*op cit*, page 213) has suggested that it may diverge slightly more than in the original reconstruction

Other talar material, notably KNM-ER 803 (Leakey, 1972 *op cit*) and KNM-ER 813 (Leakey, *ibid*, and Wood, *J Anat*, **117**, 203–204, 1973) has been attributed to *Homo* Although several fossil hominid tibia are now available from East Africa, no comprehensive study on this material has yet been completed and therefore any conclusions must remain very tentative Preliminary studies again suggest, however, the existence of two morphological, and presumably locomotive, patterns Among this material the partial skeleton, KNM-ER 1500 (including a tibia) and KNM-ER 1476 have been allocated to *Australopithecus* (Leakey, *Nature*, **242**, 447, 1973) Conversely, the partial skeleton, KNM-ER 803 (with a tibia) has been allocated to *Homo* (Day 1973, *op cit*), KNM-ER 1481 seems also to fall with this group (Leakey, 1973, *op cit*) The tibia and fibula from Bed I at Olduvai Gorge (OH 35) has been tentatively allocated to '*Homo habilis*' (Leakey, Tobias and Napier, *Nature,* **202,** 7, 1964) but in view of recent developments in the field (Leakey, *Nature,* **248,** 653, 1974) perhaps the criteria for this taxon should be re-evaluated

Thus several lines of investigation firmly suggest that a locomotor dichotomy existed within the bipedal early Pleistocene hominids Although the functional nature of this dichotomy is not yet entirely clear it may be that the striding gait of *Homo sapiens* was not present within the 'australopithecine' group (Day and Wood, *op cit*) Moreover, although some workers in the field insist on the use of a broad spectrum of analytical tools, there is a tendency in the recent literature to de-emphasise the importance of such comprehensive anatomical biomechanical and radiographic studies and to concentrate heavily if not exclusively on multivariate statistical evaluations The basic mechanism of this approach involves quantification of the specimen and then, in the case of fossil material, interpolation of a function of these measurements into a comparative matrix composed of measurements from living primate groups and perhaps other fossils This somewhat mechanistic approach, although admittedly useful as part of a broader study has important limitations when used as a total analytical approach First, some significant features of bones do not lend themselves to quantification The disposition, orientation and density of trabecular bone are such features, the development and orientation of muscular markings are others Second, such a method assumes that the locomotor pattern of the fossil has a known analogue such an assumption is unproven, especially with regard to the fossil Hominidae, since it now seems likely that the 'australopithecine' pattern was unique The hazards of this rather narrow approach were well demonstrated in a recent paper by Oxnard *op cit* On the basis of only eight parameters of the tali of TM 1517 and OH 8 Oxnard's results suggested that the australopithecine foot had "distinct morphological affinities with the genus *Pongo*"—an affinity clearly unlikely on the basis of the total morphological appearance of the nearly complete OH 8 foot

In summary investigations of the development of locomotion within the hominid lineage provides one of the more active and fascinating areas of palaeoanthropology today It is important, however, that such studies be as broadly based as possible and utilise the research tools, analytical methods and descriptive models of many disciplines and only in this way is constructive insight possible

From a Correspondent

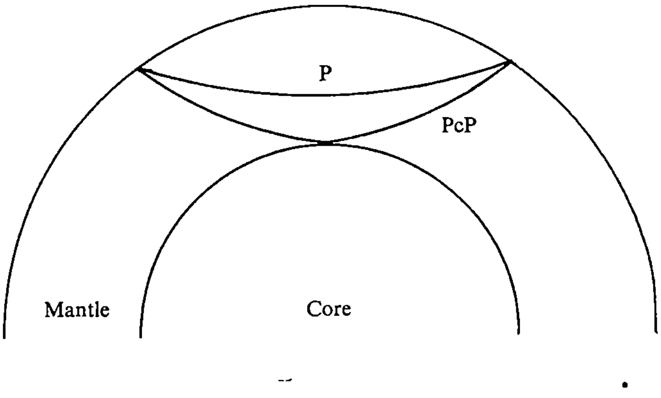
The Earth gets more complex

ONE of the major occupations of seismologists for many years has been to determine the travel time of compressional (P) waves and shear (S) waves through the deep interior of the Earth A knowledge of travel time as a function of distance from source to receiver is essential in locating earthquakes—it is also a most important ingredient in determining physical parameters of the Earth The more effort that has been expended, however, the further the goal has receded First, it became obvious that there were strong lateral variations in elastic properties (of the order of 5%) in the upper mantle, so that individual sources and receivers needed appropriate corrections Then in the past few years it has been increasingly clear that even below a depth of 700 km lateral variations persist An article by Jordan and Lynn (*J geophys Res* , **79,** 2679–85, 1974) highlights the advances now being made

It is interesting that the first authors to show that the assumption of lateral heterogeneity at great depths was unjustified did not consider travel times at all Travel times are relatively insensitive to the presence of inhomogeneities whereas the amplitude of the incoming signal and its direction of approach are much clearer indications of heterogeneity—although difficult quantities to invert for the purpose of making anything more than a qualitative statement In 1969 some observations were made of amplitudes at source–receiver distances which were well into the range in which the core should have shadowed out P waves (Phinney and Alexander, *J geophys Res* , **74,** 4967) It was clear that amplitude at a given distance was highly variable and depended entirely on which region of the core–mantle boundary was sampled in each particular instance Geophysicists are loath to consider substantial lateral variations within the core (perhaps that will change too) so there were clear indications either that the core boundary had strong topography on it or that the physical properties of the rocks at the bottom of the mantle are laterally variable—even to the extent of 5%

Then large arrays began to come into operation and results from them posed some problems, in that the angle of approach predicted by classical methods was often up to 5° away from the measured angle of approach This provoked much research from which there eventually emerged a feeling that the anomalies could not be properly explained without resort to marked lateral heterogeneity deep in the mantle (Davies and Sheppard, *Nature,* **239,** 318, 1972)

The time was ripe for another hard look at travel times and Julian and Sen Gupta (*Nature,* **242,** 443, 1973) came up with a very surprising result When every care was taken to avoid lateral heterogeneity in the upper mantle by using only very deep quakes as sources, and when the data were (as far as possible) not bunched together in a computer, from which only a mean could be extracted, significant patterns emerged By retaining a knowledge of the approximate path sampled by each data point, it was possible to see broad general regions in the deep mantle (mainly within a few hundred kilometres of the core–



Body-wave paths through the Earth's mantle

mantle boundary) out of which P waves emerged up to 1 s early or late relative to a standard travel time table Much of what in earlier studies had been regarded as general scatter arising from reading errors, crustal structure and so on was probably genuine evidence for deep lateral heterogeneity

Seismologists, one suspects, accepted the travel-time results more readily than they had the rather more circumstantial evidence from amplitudes and arrays In any case it now looked as if there was a whole new field of seismology opening up—mapping the deep mantle Jordan and Lynn's results point to a clear and fruitful way to go about it

On a seismic trace of a large earthquake many different signals can be identified and timed to a fraction of a second In addition to the P and S waves, phases called PcP and ScS are clearly visible—they are reflections of P and S waves from the core–mantle boundary If the time interval between the phases P and PcP is measured, it should, in a laterally homogeneous Earth, depend on source-receiver distance but not on anything else Furthermore, any deviations in elastic properties in source or receiver region will be sampled almost equally by the two phases, which follow paths in the upper mantle that are relatively close to each other Thus to a first approximation upper mantle anomalies are eliminated when the difference of times is taken

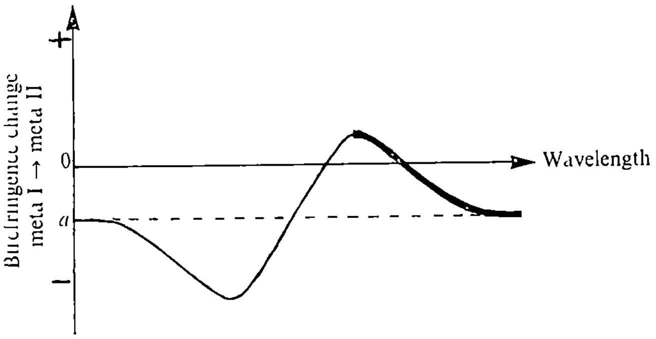
What Jordan and Lynn find is that for an earthquake in South America recorded across the North American continent, the PcP—P and ScS—S times by no means conform to standard travel time tables Along a certain azimuth in particular the differences are up to 1 s (PcP—P) and 4 s (ScS—S) away from the standard This, in seismological terms, is a significant deviation They go on to show that the anomaly most plausibly occurs along the path of the phases P and S, not the core-reflected phases, and so there is rather strong evidence for anomalous structure in a fairly narrow region at a depth of 600 to 1,400 km It is thought that the P-wave velocity is at least 1% (and probably more) greater than that of the surrounding material

Is this just a strange quirk of nature? Another stamp to go in a collection which could expand rapidly? More than that, I think Seismologists are often in danger of seeing their results as no more than information about seismic velocity in the Earth, but variation in velocity means variation in composition or temperature and that means a handle, however loose at present, on the geodynamics of the deep mantle Do plates penetrate beyond 700 km? Is the deep mantle in convection? How laterally variable in temperature is the core at its boundary? The results of the past few years are beginning to make these questions just a little more reasonable to ask with some hope of an answer in the foreseeable future

DAVID DAVIES

Birefringence and vision

IN the bleaching sequence of the visual pigment rhodopsin, the main spectral absorption shift (480→380 nm) occurs in the step between photochemical intermediates metarhodopsin I and II This is observed in both intact rod outer segment membranes (Liebman, *Biophys J*, **2**, 161, 1962) and in detergent-solubilised preparations (Hubbard, Bownds and Yoshizawa, *Cold Spring Harb Symp quant Biol*, **30**, 310, 1965) This step may involve an unfolding or other conformational change of the opsin protein which could produce the primary physiological response in vision There are several lines of evidence in support of this idea The Schiff's base bond attaching the retinal chromophore to the protein becomes chemically accessible at this step in solution (Bownds and Wald, *Nature*, **205**, 254, 1965), and probably in intact rods (Falk and Fatt, *J Physiol*, **183**, 211, 1966) There is also evidence for a conformational change in the protein on bleaching not specifically linked to the meta I→meta II step Hubbard *et al* (*ibid*, 1965) inferred a conformational change in solution from ultraviolet absorption spectra An increase in solubilised hydrodynamic volume has been observed by Heller (*Biochem J*, **7**, 2914, 1968) Circular dichroism measurements, which would be specific to protein conformational changes *in situ*, have indicated a change in solubilised pigment, but this change does not persist in the membrane (Shichi, *Expl Eye Res*, **17**, 533, 1973)



In this issue of *Nature* (see page 31) Liebman, Jagger, Kaplan and Bargoot report a study on the conformational changes in rod outer segment membranes by optical methods similar to those used on excitable membranes In the case of excitable membranes, there is a birefringence increase during the action potential (Cohen, *Physiol Revs*, **53**, 373, 1973) but in bleaching photoreceptor membranes there is a birefringence loss What do these observations mean?

The refractive index of a material is the ratio of the speed of light in vacuum to the speed of light in the material, and a birefringent substance has different refractive indices for light polarised in different planes In lipid bilayer membranes, where the hydrocarbon chains are aligned on average perpendicular to the plane of the membrane, the refractive index for the direction parallel to the chain axis is greater than that for the perpendicular direction The birefringence, Δn, is the difference between the refractive indices for these two directions $\Delta n = n_{\parallel} - n_{\perp}$ n and Δn are functions of the wavelength at which they are measured, so dispersion phenomena result Δn is greater for crystalline arrangements of the lipid chains than for more fluid, disordered ones where the chain orientation is less well defined The disk membrane is not only composed of lipid, however about half the volume is protein, which may also be anisotropically arranged The birefringence resulting from molecular organisation is called intrinsic birefringence, and that arising from the stacked arrangement of rod outer segment membranes is known as form birefringence The Δn of rods, observed to be positive, is the sum of the form birefringence, negative for a pile of parallel sheets, and the intrinsic component, which must therefore be positive and larger in magnitude than the form component (Schmidt, *Kolloidzeitschrift*, **85**, 137, 1938) It is the intrinsic Δn which tells us about the molecular orientations in disk membrane, although it cannot distinguish between the membrane constituents Form and intrinsic contributions to Δn can in principle be separated, if $n_{\text{cytoplasm}}$ can be varied independently of n_{membrane}, until the form component is eliminated This requires that the methods used to vary $n_{\text{cytoplasm}}$ have no effect on the membrane spacing, structure or thickness

Liebman *et al* first recorded a birefringence spectrum of dark adapted rods in the visible region, then followed the changes in Δn during bleaching They found a birefringence change appearing at about the rate of the metarhodopsin I→II step, the 'birefringence transient' (BRT) The figure is a schematic diagram of the fast birefringence change plotted against wavelength This curve can be fitted by the sum of two components, one resulting from the optical effects of bleaching, and the other directly caused by changes in the membrane The first component can be calculated theoretically, and it has the shape of the curve shown, but its position is shifted upwards so that it is centred on the axis at zero BRT The observed change is indicated by the heavier portion of the curve Once the effect of pigment absorption loss is accounted for, what is left is simply a constant (over the wavelength band measured) birefringence loss This birefringence loss is attributed to a change in the intrinsic birefringence of the membrane As in the case of nerve, there are many ways of explaining this change in terms of molecular events It could be due to any combination of small reorientations of lipid or protein, or slightly increased disorder Nevertheless, Liebman *et al* have detected a small change which may signal the start of the visual impulse

HELEN SAIBIL

Astrophysics and energy from black holes

EVER since Einstein's discovery of the famous equation $E=mc^2$ the direct conversion of mass into energy has remained man's most hopeful source of power Atomic fission reactors, and a possible future generation of fusion devices, manage to achieve the remarkably good efficiency (by terrestrial standards) of converting about 1% of nuclear rest mass into energy But there almost certainly lurks in the Universe a source of mass conversion with a theoretical efficiency of up to 100% This source is the famous spinning black hole

It is now generally appreciated that black holes may only be characterised by three parameters, mass, electric charge and angular momentum Other than this all black holes appear to the outside world to be identical, whatever their internal constitution Black holes with rotation possess a number of strange properties which are not shared by their nonrotating counterparts For example, suppose an inquisitive explorer were to take a trip in a rocket ship near the surface of a rotating black hole As he approached closer, he would observe that the effect of the enormously compact mass was to try to drag his rocket around with it as it rotated More and more fuel would need to be expended to resist this effect until, at a certain distance from the object, no amount of rocket power in the universe could prevent the ship from being dragged around in circles The explorer has entered a new region, the ergosphere, where it is impossible to be 'at rest' Inside the ergosphere is an event horizon, which if crossed will forbid an object to leave again Anything which arrives inside this horizon cannot by any power available prevent itself from falling towards the centre of the black hole But an object which has only ventured as far as the ergosphere can not only escape to the outside world again but actually do so with the bonus of more energy than it went in with Hence the name 'ergosphere'

The precise details of this energy-generating process were discovered by Penrose in 1969 and are based on the fact that a particle in the ergosphere is able to move along a trajectory which has negative total energy relative to a distant observer Consequently, if a particle falls into the ergosphere and breaks into two pieces such that one of these pieces moves on a negative energy trajectory, then the other piece will re-emerge with an energy which is greater than the original combined energies of both pieces, while the sacrificed piece will plunge on into the hole, the negative energy it carries thereby reducing the total mass and angular momentum of the black hole The process cannot, however, be repeated indefinitely so that the black hole disappears altogether Eventually, when the angular momentum has been reduced to zero, no further energy may be extracted in this way

A rather fanciful practical application of the Penrose process has been discussed by Misner, Thorne and Wheeler (*Gravitation*, 908, Freeman, San Francisco, 1973) An advanced civilisation could build a city around a rotating black hole, and daily dispose of its waste by dropping it in special vessels into the ergosphere, where the waste would be tipped out along a negative energy trajectory, thereby not only obligingly disposing of itself in the most effective dustbin in the Universe, but in addition supplying the re-emerging vessel with an energy equivalent of the entire waste rest mass, plus some of the rest mass of the black hole as well

As well as contributing to the solution of cosmic ecology problems, the Penrose process might serve as a mechanism for producing high velocity jets in various astrophysical situations A black hole at the centre of a galaxy could act as a repository for nearby stars, which might fragment appropriately on their way through the ergosphere, thereby imparting a very high velocity to one of the fragments, which would then emerge in the form of a jet

Strict limits on the energetics of jets formed in this way have been deduced by Wald, of the University of Maryland, in the *Astrophysical Journal* (**191**, 231, 1974) The argument used involves a simple comparison of the breakup process in ordinary special relativity with that occurring in the ergosphere of a rotating black hole

The results of this comparison are disappointing for the astrophysicist So similar are the limiting expressions in the two cases that the energy of the emitted fragment which is attainable is not greatly different whether the black hole is there or not For example, an object simply falling from rest a great distance away could initiate the ejection of a fragment with a velocity at most $1+v\sqrt{2}$ compared with the special relativistic value of v The emitted fragment can never be relativistic unless the incident body or the breakup velocity are themselves relativistic

Thus, although rotating black holes may well be responsible for triggering violent explosions at the centres of galaxies, it seems that the Penrose energy is unlikely to be making a major contribution to such events after all

P C W DAVIES

Hunt the helix

from E G Richards

No sooner was the first primary structure of transfer RNA worked out, than the clover leaf model for its secondary structure was proposed This has become an article of faith for molecular biologists and all of the 60 sequences now available conform to it When the sequence of 5S RNA from *Escherichia coli* was published in 1967, several authors leapt to the challenge and published models for its secondary structure although computer investigations suggested that there were several million possibilities The favourite tack today is to devise experiments that eliminate at least some of these Kearns and Wong (*J molec Biol*, **87**, 755–774, 1974) have attempted to go one better and deduce a unique structure from the application of the nuclear magnetic resonance (NMR) method which they and others have so successfully applied to tRNA structure

Resonances in the range 12–15 p p m arise from hydrogen bonded ring N–H protons from U or G so that the number of resonances gives the number of AU and GC pairs—at least it does if GU pairs are ignored since this pair has two H-bonded ring N–H protons, but this possibility is glossed over Kearns and Wong find that at 35° C there are 28 ± 3 base pairs on this basis of which but 4 are AU As the temperature is increased about 10 resonances have melted out at 52° C to leave a 'core' structure which is stable to 62° C By 73° C a further 9 or so have gone to leave about 9 base pairs This maximal figure of 28 pairs is at variance with estimations obtained from the analysis of various optical properties which are in the range 38–44 pairs These optical methods are fraught with assumptions that are difficult to test adequately and it is reasonable to accept Kearns and Wong's result as being the more reliable

Kearns and Wong have attempted to delineate the precise base pairing scheme involved To do this they have employed explicit semi-empirical rules for calculating the precise shift expected of an AU or a GC base pair in a variety of different helical environments defined by its nearest neighbours They thus show that the structure left at 73° C gives a spectrum in close agreement with that expected of the longest and presumably most stable helical region that it is possible to form from the sequence They have gone on to list all possible helical regions in order of length and GC content and worked out the position of the resonances of each They thus show that the 'core' present at 52° C could contain the two most stable helices, H$_1$ and H$_2$ If these two are present, H$_3$–H$_{10}$ are automatically excluded since they each involve residues in common with the first two, moreover, H$_6$–H$_{10}$ give resonances that are inconsistent with the 52° C spectrum The next most stable contender is H$_{11}$ and the spectrum comprised of the H$_1$, H$_2$ and H$_{11}$ contributions is in nice agreement with the 52° C result and they conclude that the core contains these regions This core structure is of some interest in that is essentially three dimensional unlike most proposals for RNA secondary structure which can be neatly drawn on a flat page of a journal without the chain crossing itself—a restriction that would seem to be purely aesthetic in origin

The helices comprising the core rule out all but 12 of the remaining possible helices and calculation of the spectra of these eliminates all but 6 from further consideration Not all combinations of these 6 are possible but the NMR data do not allow further progress Kearns and Wong thus invoke the results of oligonucleotide binding experiments that imply that certain regions of the sequence are non-helical This serves to eliminate all but H$_{17}$ and H$_{79}$ They thus provisionally conclude that the full low temperature structure contains the five helical regions H$_1$, H$_2$, H$_{11}$, H$_{17}$, and H$_{79}$ giving a total of 28 base pairs of which 11 are AU The discrepancy in the number of AU pairs is perhaps not too serious in that the partition of the low temperature spectrum into AU and GC regions is subject to error The question may be raised as to whether this structure corresponds to the native or denatured forms of the molecule Kearns and Wong claim that their results are independent of the nature of the solvent and the mode of preparation suggests that at least some of their solutions contained a mixture of both forms It may be that the two forms are not distinguished by their technique even though it is almost certain that they have different base pairing schemes

A further question is whether their final model is compatible with other available experimental data which they do not invoke The results of limited nuclease attack have been interpreted to imply that certain pairs of regions of the structure are not hydrogen bonded to each other but, alas, it can be readily shown that Kearns and Wong's model is incompatible with these interpretations Maybe these assumptions are at fault or maybe Kearns and Wong were too simplistic in ignoring GU pairs, looped out bases and other possible structural complications Enthusiasts for these sorts of games await further developments with eager anticipation

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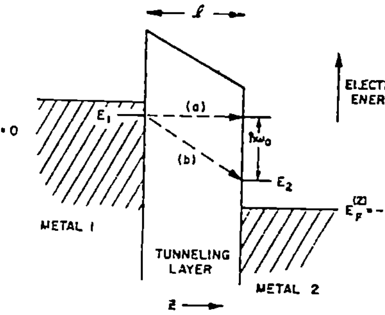
Solid state test tubes

from our Solid State Physics Correspondent

MANY solid state scientists have wondered whether the particular physical system with which they work (possibly a semiconductor surface or a glassy matrix) might be so sensitive to the activity of added chemicals that they could identify the chemical precisely by means of a simple electrical measurement on that solid The result would be a novel way of following reactions occurring in or on a solid—a sort of solid state test tube

One example of the fulfilment of such a wish has been developing over the last few years Giaever discovered in 1960 that the I–V characteristic of metal–insulator–metal (MIM) sandwiches was controlled by tunnelling if the insulator was only 20 Å thick He realised that the non-ohmic behaviour of the I–V characteristic happened because of the transitions of the tunnelling electron from an energy level in one metal to a different level in the other and that energy-band information —for example the size of a superconducting energy gaps—could be extracted from these I–V characteristics In fact, the I–V curve not only contained band information but also showed fine structure arising from phonon transitions and other vibrational processes (Jaklevic and Lambe, *Phys Rev Lett* **17**, 1139, 1966) In Pb–Al$_2$O$_3$–Al sandwiches an Al phonon transition can be identified and, if water is present, then the effect of OH bond vibrations are seen The reason is that the tunnelling electron, driven by the applied field, can either make a loss-free transition to a state of the same energy on the opposite side of the barrier (E_1 in the figure) or it can make a transition to a lower level, E_2, provided it can lose exactly the right amount of energy ($\hbar\omega_0$ in the figure) This energy can be transmitted to the O–H group by inelastic transfer and the transition provides an increase in the 'inelastic tunnelling current' at a potential corresponding to $\hbar\omega_0$



It must then have occurred to Lambe

and Jaklevic "If it works with the water impurity, why not with other impurities doped into the insulator lattice or even simply spotted into the surface?" Surprisingly, organic impurities did indeed couple well with the tunnelling electrons and measurements of the fine structure of the I–V curve, with very sensitive amplification and differentiation, yielded a spectrum of the bending, rocking and stretching bands of organic groups which was a near perfect analogue of the infrared or Raman spectrum with only a few special solid state effects to be accounted for Lambe and Jaklevic demonstrated this for simple volatile organic liquids such as acetone and showed that one monolayer of molecules gave suitably strong peaks but that, for sharpness, the sample must be cooled well below the temperature of liquid nitrogen (*Phys Rev*, **165**, 821, 1968) The peaks seen in the plot of d^2I/dV^2, where I is the tunnelling current for an applied voltage V, correspond very closely to the infrared absorption peaks if the voltage scale is regarded as an energy scale in eV For example, the peak of the O–H bending band appears in both measurements at precisely 0 118 eV

Simonsen and Coleman, of the University of Virginia (*Nature*, **244**, 219 1973) then developed a routine system for depositing thin layers of many organic solids by evaporation, using a special apparatus which allowed all MIM device fabrication and sample deposition to be done during one vacuum pump-down At this point, it was certainly true to say that a very original, special and useful method of vibrational spectroscopy had been added to the infrared and Raman methods A crude 'solid state test tube' had been developed which would yield a vibrational spectrum without costly infrared optics

More compounds were, of course, applied to this test cell And it must be said that, as work progressed, the doubts of the sceptics were less and less justified Within the range 0 03 to 0 55 eV (300 to 4,400 cm^{-1}) all the correct bands turn up Coleman and Hansma (*Science*, **184**, 1369, 1974) have now extended the work to some large organic compounds which can only be applied from solution The success of this 'solution doping' is a breakthrough in that very many more compounds can now be studied and, as a speculation, perhaps reacting solutions can be applied to the film and reaction products identified by the vibrational spectra Since only a monolayer is required and the MIM devices are 1 mm square, the arrangement can be operated with sub-microgram quantities of material Of course, the compounds must survive the process of drying on to an anodic Al_2O_3 film and the subsequent evaporation of a thin overlying metal film Even large molecules such as calf thymus DNA and *E coli* RNA seem, however, to survive this treatment since the tunnelling spectra exhibit the expected phosphate vibrations, ring distortions and other purine, pyrimidine and sugar group vibrations There must, in some cases, be surface reactions and, at the very least, frequency shifts because of absorptive forces and the effects of cooling These effects will then limit the delicacy with which the method can be used as an absolute sensor of bond vibrations and may also exclude the examination of easily altered compounds such as polypeptides (there is ominous silence on this class of compound)

Thus the inelastic electron tunnelling method, although still requiring much development, is probably the first of a whole range of techniques in which the behaviour of carriers in solids is used to recognise chemical characteristics of juxtaposed molecules by quantum mechanical coupling

Competition in the laboratory

from a Correspondent

IT is common knowledge that an ecologist's view of nature is coloured strongly by the particular species he or she works with Mammologists emphasise the role of aggression, ornithologists the importance of dispersal ability, and entomologists the extinction of local populations One example of this phenomenon appears in discussions of competition between species Some ecologists have argued the coexistence of two species on a single limiting resource is impossible (Gause's Exclusion Principle) and have supported this view with laboratory experiments (Gause, *The Struggle for Existence*, Dover, New York, 1958)

Others have rejected Gause's Principle as being unnecessary and have supported their claim also with laboratory experiments (Ayala, *Nature*, **224**, 1076 1969) Wallace (*Ecology*, **55**, 245, 1974), in the most extensive series to date of experiments on animal competition in the laboratory, has shown how difficult it is to subscribe to any single view of competition, even when considering an apparently similar group of species in the simplest possible conditions

Wallace has examined in detail the competition within and between eight species of *Drosophila* maintained on laboratory medium Although there is no reason to assume that the species are very different in their ecological requirements, Wallace found several different types of interactions Some pairs interfered with each other, as would be expected, but others appeared not to interact and others even to facilitate each other, permitting each to maintain higher numbers than it could alone When there was interference, it sometimes affected both species and sometimes only one of the pair

Wallace's experiments make it difficult either to accept or reject Gause's Principle Although it is probably true that two species cannot coexist on a single resource, Wallace's results clearly indicate that, in advance, it is impossible to decide what constitutes a single resource To describe the competition between several pairs of species, an individual of one species could not be replaced by a fractional equivalent of the other species That undermines the basis for much of the theory which supports Gause's Principle (MacArthur, *Geographical Ecology*, Harper and Row, New York, 1972) Certainly the construction and application of predictive models of competition between species which are based on the existence of a single limiting resource will prove to be very difficult unless competition in nature is considerably simpler than in Wallace's laboratory

Self protection in neuromuscular transmission

from our Insect Physiology Correspondent

DURING recent years there has been accumulating evidence that L-glutamate serves as the neuromuscular transmitter substance of arthropods, taking the place of acetylcholine in vertebrates in this respect The evidence has not yet convinced all authors some still maintain that glutamate is merely a non-specific agent which will depolarise muscle fibres and ultimately desensitise the receptor sites One of the unsolved problems lies in the failure of the muscles in arthropods to be affected by the L-glutamate normally present in the blood How this is prevented has remained something of a mystery

Murdock and Chapman (*J exp Biol*, **60**, 783, 1974) have made quantitative measurements of the concentration of L-glutamate in the blood plasma of the crayfish *Astacus*, the tarantula *Dugesiella* and the locust *Locusta* In the crustacean and in the spider this concentration was found to be so low that even tenfold the normal level, applied in saline, had no effect on neuromuscular transmission although higher concentrations did cause depression On the other hand the average concentration of L-glutamate in *Locusta* is sufficient to depress transmission; and slightly increased concentrations, which may occur naturally, will cause marked depolarisation. The authors discuss various possible explanations of the indifference of the locust to its circulating glutamate.

In the same issue of the journal (*ibid.*, 673) Clements and May investigate this problem in great detail in the locust *Schistocerca*. They could obtain no evidence of sequestration of glutamate by haemocytes, or of binding of glutamate by haemolymph proteins. On the other hand divalent ions, calcium and magnesium, in the haemolymph do certainly form stable complexes with amino acids and the authors estimate that the level of free glutamate is probably reduced about 25% by binding to the divalent metal ions. Both increasing the calcium concentration in the test saline, and introducing magnesium ions, made nerve–muscle preparations less sensitive to glutamate. Furthermore, these authors consistently found that isolated preparations of the retractor muscle of the claw were much more sensitive to glutamate when dissected out than when perfused in the uninjured femur. The dissected preparations studied in sections showed that the connective tissue sheath of the muscle fibres was disrupted, and ready access given to the spaces between the fibres where the neuromuscular junctions lie. They conclude that the connective tissue sheath serves as protective barrier against glutamate in the haemolymph.

Clements and May are inclined to accept the view that glutamate is normally responsible in insects for the transient depolarisation of the postsynaptic membrane that follows nervous stimulation. But, as Murdock and Chapman point out, even if it does not have this physiological role, the neuromuscular junctions must be protected from the action of unwanted glutamate.

Oscillating yield in a plastic

from Robert W. Cahn

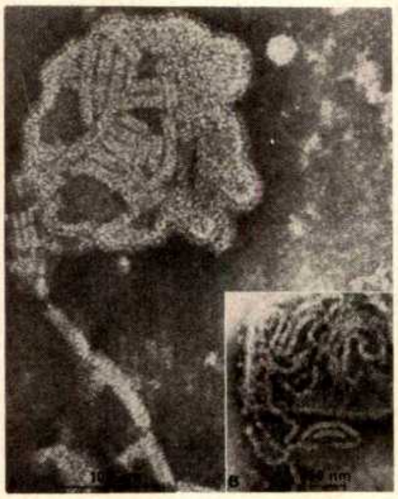
SOME years ago, a group of Russian polymer scientists (Andrianova *et al.*, *J. Polym. Sci.*, *A2*, **9**, 1919; 1971) discovered a novel form of behaviour in several polymers. The neck which forms during tensile straining of the amorphous polymer turned out to be made up of successive bands of translucent and opaque material, and the stress–strain curve showed corresponding strain oscillations. The opaque material had evidently crystallised.

This phenomenon of 'oscillating necking' has been theoretically analysed by Barenblatt in the Soviet Union (*Mekhanika Tverdogo Tela*, No. 5, 121; 1970) and to back up this analysis, Roseen of AB Atomenergi in Sweden has adopted an unusual experimental approach (*J. Mater. Sci.*, **9**, 929; 1974). He examined a tensile specimen of amorphous PETP during tensile deformation by means of an infrared Thermovision camera, which records instantaneous temperature distributions. Strips of high temperature (up to 95° C) develop in association with the opaque crystalline strips. The calibration and the resolving power of this camera are set out in some detail. It seems, therefore, that during oscillating necking, a narrow layer of crystalline material forms internally (micrographically it was shown that the surface remains amorphous). Crystallisation seems to be the result of rapid local deformation, which adiabatically heats a narrow front of polymer. The crystalline material is much less ductile than the amorphous matrix; plastic flow and its associated heat generation slows down the stress rises and the cycle repeats.

Thus, polymers may show behaviour superficially similar to the discontinuous yielding long familiar in certain alloys. This is known as the Portevin-Le Chatelier effect and is most familiar in mild steels. In essence, it is caused by the cyclic locking of dislocations by readily mobile impurity atoms (normally carbon or nitrogen). A group of dislocations breaks free, the impurity atoms catch up and lock them afresh, and the cycle repeats.

Roseen has confirmed the postulated difference in tensile behaviour between amorphous and crystalline polymer by examining the effect of intentional heat treatment of the polymer on the form of the tensile curve. Here is another unfamiliar point of similarity between metals and polymers: it is second nature to a metallurgist to examine the effect of systematic heat treatments on the mechanical behaviour of an alloy, but this is not yet a normal experiment for a polymer scientist.

One lesson from this intriguing piece of work is that it can be illuminating for a polymer scientist to know something of physical metallurgy. But it would be hubris to leave it at that. Increasingly, metallurgists have something to learn from polymer scientists, as concerns both phenomena and experimental techniques. It is time that 'polymer crystallisation' became a normal part of the syllabus of students of metallurgy and materials science.



Viruses naked and clothed

Two views of paramyxoviruses. A, Newcastle disease virus, a pathogen of chickens. The protein envelope has been broken to allow the escape of part of the nucleocapsid. B, Nucleocapsid from Sendai virus, the favourite of those who fuse cells. The nucleocapsid in both viruses consists of one long single-stranded RNA molecule around which identical protein subunits are arranged. The periodicity of the helix thus formed is visible in B. Photograph from *Virus Structure* by Robert W. Horne (Academic Press, New York and London, 1974).

Frenkel pairs in zinc selenide

from John Walker

To account for the electrical conductivity observed in some ionic solids, Frenkel suggested in 1926 that a small percentage of ions could leave their lattice sites and migrate through the crystal. The point defects so formed, the lattice vacancies and their corresponding interstitial partners, have hence been called Frenkel defects. The concept has been widely used during the past 48 years, but until recently no Frenkel defect had been observed directly. Watkins (*Phys. Rev. Lett.*, **33**, 223; 1974) has now remedied the deficiency.

After irradiating zinc selenide with 1.5 Mev electrons at a temperature of 20 K, Watkins observed a family of similar spin resonance spectra which he labelled V, V^{I}, V^{II}, V^{III}, V^{IV}. The V spectrum had previously been assigned to a hole localised on one of the four selenium neighbours to a zinc vacancy. By mechanically compressing the crystal Watkins found that he could make the hole jump freely between the four selenium atoms. (In the language of the trade, a [111] uniaxial stress makes that particular Jahn-Teller distortion energetically unfavourable and the defect flips over into one of the other ⟨111⟩ orientations.)

The other spectra are so similar to V that Watkins also assigns them to zinc vacancies. But the V^I centres do not reorient under uniaxial stress. They are therefore locked in position in the strain field of a nearby defect—the zinc atom which has been ejected to create the vacancy. It occupies the interstitial site nearest to the vacancy.

Warming the crystal above 20 K causes the interstitial zinc atom to move to a new site, behind one of the four selenium atoms surrounding the vacancy. This configuration, called V^{II}, is of lower energy than V^I because the polarisable selenium atom is between the vacancy and the interstitial. In V^{III} the interstitial lies in the ⟨100⟩ direction. No model was proposed for V^{IV}, the spectrum of which is very weak.

Directional effects observed during irradiation confirm the models. For example, when the electron beam direction is $[\bar{1}\bar{1}\bar{1}]$, more V^I defects are observed in the $[\bar{1}\bar{1}\bar{1}]$ configuration than in other ⟨111⟩ orientations.

Another interesting observation is that the isolated vacancy V is not produced linearly with electron dose. The creation rate follows a square (or perhaps a cube) law. This suggests a two-stage process, the formation of close-pair Frenkel defects being the first step.

The direct observation of vacancy–interstitial pairs in a solid is an interesting confirmation of Frenkel's original suggestion, although its impact is perhaps lessened by our present awareness of the richness and complexity of point defects created by irradiation. (An awareness due in great measure to Watkins, one might add.)

The lessons for *aficionados* of spin resonance are twofold; look for these Frenkel defects in other materials; and use the uniaxial stress technique, without which their identification in zinc selenide would have been much more difficult.

Components of the amoeba membrane

from a Correspondent

FASCINATING details of the surface structure of some primitive animal cells, amoebae and sea anemones, have recently come to light. They provide evidence for new classes of polyanions that may serve functions in primitive organisms, similar to those performed by glycoproteins containing sialic acid and the gangliosides and proteoglycans of metazoal cell surfaces.

Korn and his colleagues have been studying *Acanthamoeba castellanii*, a small free living soil amoeba that grows well on a soluble medium. About a third of the membrane is made up of a unique component that is extracted into solution with 8 M urea, or better, aqueous butanol, and is easily purified free of a non-glycosylated protein fraction. The new component contains neutral sugars, mainly mannose glucose and xylose, about 14% of fatty acids that include unusual branched chain 2-hydroxy fatty acids, and aminophosphonic acids. Of these last derivatives, the parent compound but not 1-hydroxyl-2-aminoethyl phosphonic acid are known constituents of lipids in various species of lower marine invertebrates but perversely not to any significant extent in amoebae.

Clearly the lipophosphonoglycan described by Korn and his colleagues Dearborn and Wright (*J. biol. Chem.*, **249**, 3335-3341; 1974; **249**, 3342-3346; 1974) is an integral component of the membrane and presumably is anchored into it by association of the fatty acids with the phospholipid-sterol matrix of the membrane. Brief mention is made of unpublished electron microscopic evidence by Bowers and Korn that the polar moieties of lipophosphonoglycan including phosphate groups and concanavalin A reactive sites (α-mannosyl groups) are exposed on both sides of the amoeba surface membrane.

The exact structure of this polymer is unknown. If the sugars are in normal glycosidic linkage, a core structure of galactosamine residues and aminophosphonic acids, both perhaps substituted with fatty acids and forming the attachment sites for the neutral sugars, seems to be most likely. The linkages between the constituents of the 'core' region are not known except phosphoamide bonds are excluded since these would be labile to acid hydrolysis and this is not observed.

In another publication Allen *et al.* (*J. Cell Biol.*, **60**, 26–38; 1974) describe somewhat similar material at the surface of a uninucleate, freshwater phagocytic amoeba T_1D_{13}. The only anionic group detected by Allen *et al.* at the cell surface is phosphate which accounts for more than 10% of the isolated surface membrane. Most of this phosphate (90–95%) has been isolated as a polymer in association with *neo*-inositol. Amino phosphonic acids were not detected. The large sugar content, mainly mannose rhamnose and glucose, of these plasma membranes was isolated separately in an uncharacterised fraction that seems to contain appreciable organic phosphate. It seems that these two fractions may be more closely related in the native structure, and perhaps are hydrolytic fragments produced during isolation (a part of the phosphate is very acid labile according to Allen *et al.* whereas the remaining phosphate is much more stable; the pronounced stability to acid hydrolysis of C–P bands as in phosphonic acids is a characteristic of the Korn polymer). Some structural similarity between the undegraded phosphorylated polymers of *Amoeba* T_1D_{13} and *Acanthamoeba* cannot therefore be totally excluded particularly since the analysis of Korn and his associates for lipophosphonoglycan purified from *Acanthamoeba* still leaves substantial leeway for inclusion of a polyol such as *neo*-inositol.

The clearest existing analogy to the amoeba lipophosphonoglycan is perhaps the lipopolysaccharide of certain Gram negative bacteria. Both sorts of compounds contain fatty acids including hydroxy fatty acids, and neutral and aminosugars. The bacterial polymer contains ethanolaminephosphate, the ester analogue of 2-aminoethyl phosphonate. The fraction isolated by Allen *et al.* perhaps suggests superficially a resemblance to another ubiquitous class of bacterial polymers, the teichoic acids which are based on ribitol or glycerol phosphate derivatives. Of course, it may turn out that the amoebae polymers are unique structures with no known biological precedents.

Aminophosphonic acids also turn up in phosphonoglycoproteins isolated from *Metridium dionthus* (Hilderbrand *et al., Biochemistry*, **12**, 4756–4762; 1973). These partially purified fractions contain 2-aminoethyl phosphonic acid, neutral sugars and hexosamines but apparently not significant amounts of fatty acids. The structural relationship of these polymers to the lipophosphonoglycan and in particular the method of attachment of the chains containing organic phosphorus to the polypeptide moiety will be of interest.

The prevalence of surface negatively-charged polymers of diverse structure in the lower invertebrates suggests a positive function in the ecological success of these organisms. This role may be as a mechanism providing a stable environment surrounding the cell, and may be related to the ability of the amoebae surface to absorb large quantities of various cations. This function in turn has relevance to subsequent pinocytotic events during feeding and in the uptake of nutrients under culture conditions. The existence of an anionic surface that could function as a buffering system could also form a valuable protective role against foreign cationic substances present in the surrounding medium (sea water). Alternatively, if present in the buccal cavity the phsophonate compounds may protect the organism from self dissolution by degradative enzymes spilled over from the digestive tract.

articles

Jupiter after Pioneer: a progress report

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"We now come to a much more magnificent planet, Jupiter, the largest of them all " So wrote Sir John Herschel in A Treatise on Astronomy[1] *Now, after a decade in which space programmes have concentrated on the inner planets, a spacecraft has been sent to Jupiter This article describes how the data sent back are changing our ideas about the planet*

In December 1973, Pioneer 10 became the first artefact of man to reach the environs of Jupiter In the two weeks that it spent travelling through the Jovian magnetosphere, it sent back more than 300 pictures of Jupiter, as well as measurements of magnetic fields, energetic particles, plasma, infrared and ultraviolet emissions, and micrometeoroids The spacecraft passed within 1 8R_J of the cloudtops of Jupiter (R_J, the radius of Jupiter, is 71,600 km) Preliminary reports have now been published[2–18], and here I present a progress report summarising them, describing some of the first theoretical interpretations, and suggesting some of the gaps that remain in our understanding of Jupiter

Radio occultations

The temperature structure of the Jovian atmosphere over more than 100 km of altitude has been inferred by Kliore *et al*[16] from a study of the Pioneer S-band (2,200 Mhz) signals as the spacecraft was occulted by Jupiter (see Fig 1) They found the temperature to behave quite differently from all published models, with a very strong temperature inversion at about 20 mbar pressure and 280 K temperature, which may be explained by absorption of solar radiation by dust[16,19] They also expect to derive an electron-density profile of the Jovian ionosphere, but it is complicated by multipath propagation[16] This will be a welcome addition to the many ionosphere models that have lacked any data by which to be tested

Pioneer's trajectory was designed so that it would be occulted by the Jovian satellite Io It had been suspected for several years that Io might have an atmosphere, because Io is sometimes brighter after passing behind Jupiter as if snow had fallen during the passage through Jupiter's shadow[20–22] Recently, evidence of atomic sodium gas at Io has been found by R A Brown[18,23] Analysing the Io occultation, the Kliore group discovered that Io has an ionosphere, with a peak density of 6×10^4 cm^{-3} at an altitude of ~100 km (see Fig 2) They estimated theoretically that this could imply a neutral atmosphere with a density at the Io surface of 10^{-8} to 10^{-10} bar They propose that Io has a new kind of ionosphere, reaching from the surface up to 700 km

Ultraviolet photometer

A year ago it was predicted that if any of the Galilean satellites have atmospheres, gases escaping from them could form large toroidal rings around the planet[24,25] Judge and Carlson[11] using Pioneer's ultraviolet photometer, found Lyman-α emissions (attributed to atomic hydrogen) of several hundred rayleighs intensity from the equatorial plane of Jupiter at a time when neither Jupiter nor its satellites were in the field of view, and they tentatively interpret the radiation as originating in such a toroid They also made the surprising discovery that Io seems to have a Lyman-α brightness of 10^4 rayleighs, corresponding to a column excitation rate of 10^{10} photons cm^{-2} s^{-1}, an order of magnitude brighter than Jupiter at this wavelength Although such Lyman-α radiation is usually the result of scattering of solar ultraviolet by atomic hydrogen, they

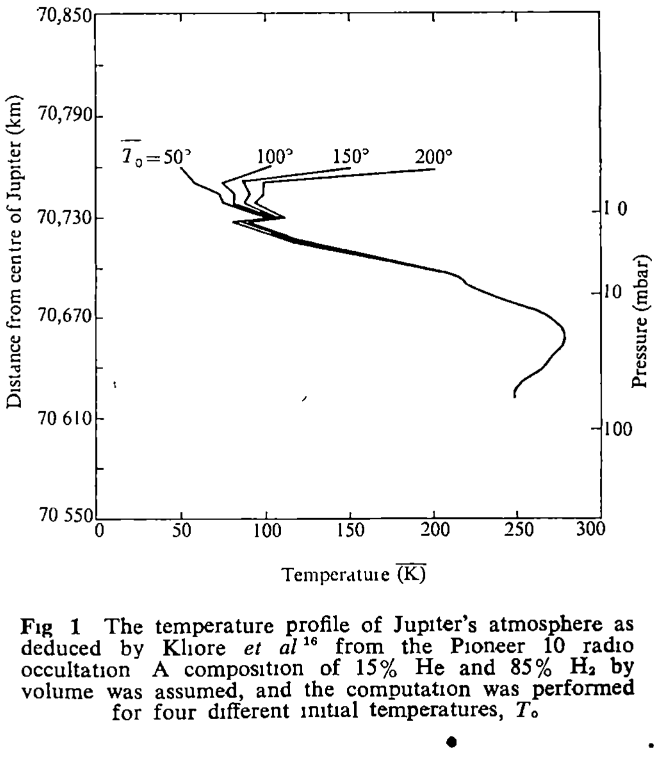

Fig 1 The temperature profile of Jupiter's atmosphere as deduced by Kliore *et al*[16] from the Pioneer 10 radio occultation A composition of 15% He and 85% H_2 by volume was assumed, and the computation was performed for four different initial temperatures, T_0

suggest that this extraordinary brightness may be due to auroras on Io

The presence of hydrogen on Jupiter has long been known, but helium had never been detected until Pioneer's arrival Judge and Carlson's photometer detected ~15 rayleighs of the He 584 Å emission line Their Lyman-α (1216 Å) channel measured a Jovian brightness of about 10^3 rayleighs, attributed to the glow of atomic hydrogen, a lower value than sounding-rocket measurements of Rottman *et al*[26] Derivation of the He/H ratio from the ultraviolet measurements awaits detailed model-atmosphere calculations, but the 1971 model of Carlson and Judge[27] suggests, for the observed helium emission, a value of He/H_2 ~ 50% in the lower atmosphere

Infrared radiometer

Chase *et al*[10] (G Munch, principal investigator) mapped Jupiter at wavelengths of 20 and 40 μm, with the Pioneer infrared radiometer The 20 μm channel contains the rotational component of the H_2 spectrum, and the 40 μm one the translational component The latter is dependent on the partial pressure of He, so for the first time it is possible to measure the He/H_2 ratio on Jupiter, although the ratio is somewhat model-dependent Theoretical estimates of the abundance of Jovian helium have ranged from the cosmic abundance, in which He/H~10% by number, up to a model in which helium is the major constituent and hydrogen a minor one[28] This abundance is an important input to models of the Jovian interior and atmosphere The Chase group calculates He/H_2 ratios (by number) from 0 6 to 0 8, that is, He/H is from 0 3 to 0 4, somewhat higher than most theoreticians expected, and higher than the values emerging from stellar occultation studies (L Wasserman, private communication)

Measured infrared brightness temperatures ranged from 115 K to 145 K, and seem to be roughly correlated with the visible belts and zones For the first time ever, the rear of Jupiter was observed, and, as expected, the planet was found to radiate as much infrared from its dark side as from the front Chase *et al* compute that Jupiter radiates about twice as much energy as it receives from the Sun, a result somewhat smaller than the aircraft measurements of Aumann *et al*[29] but removing at last any uncertainty about the far-side emission Jupiter radiates like a feeble star

Imaging photopolarimeter and television

The imaging photopolarimeter of Gehrels *et al*[12] contained a 2 5 cm telescope which made maps of Jupiter in blue and red light, polarised and depolarised, as the spacecraft spun Some measurements were also made of the Galilean satellites The most striking early results were the hundreds of photographs that resulted from the spin-scans of the planet, giving higher resolution pictures of the planet than had previously been possible Four "small" (4,000 km) bright spots have been observed, as have several bright swirls Two of the light-coloured zones of Jupiter show a distinct wave pattern The border of the Great Red Spot is dark, and sharp to the limit of resolution

Gehrels *et al* found that the blue polarisation varied from several per cent at the centre of the disk to ~40% at the poles These high polarisations are believed to be due to Rayleigh scattering, and Gehrels *et al* expect to use them to measure the amount of gas above the clouds It may be possible to derive the size, shape, and refractive index of the cloud particles now that we have measurements over the large phase angles impossible to obtain from Earth

The magnetometer

The first surprise from Pioneer was the detection of Jupiter's influence on fields and particles well before the expected time It is known that the solar wind is deflected by the Earth's magnetic field, forming a bow shock, and straightforward scaling implied that the Jovian bow shock should occur at about 50 R_J from the planet[30] But the helium vector magnetometer of Smith *et al*[5] detected the bow shock at 108 R_J The magnetopause was observed at 96 R_J, and the magnetic field remained near 5×10^{-5} gauss from there to about 50 R_J with frequent irregular dips to values near 1×10^{-5} gauss

After 25 R_J, the field began to appear more dipolar Using the field values measured when close to the planet, the experimenters estimate the dipole moment of Jupiter to be 4 0 gauss R_J^3 The dipole is tilted by ~15° and displaced by about 0 2 R_J from the centre of the planet, which implies surface fields from ~2 to ~12 gauss if quadrupole terms are negligible This is a more tilted and slightly weaker field than had been deduced from terrestrial measurements of the decametric noise bursts, but is not in serious disagreement The field is upside-down with respect to the Earth's which was also expected

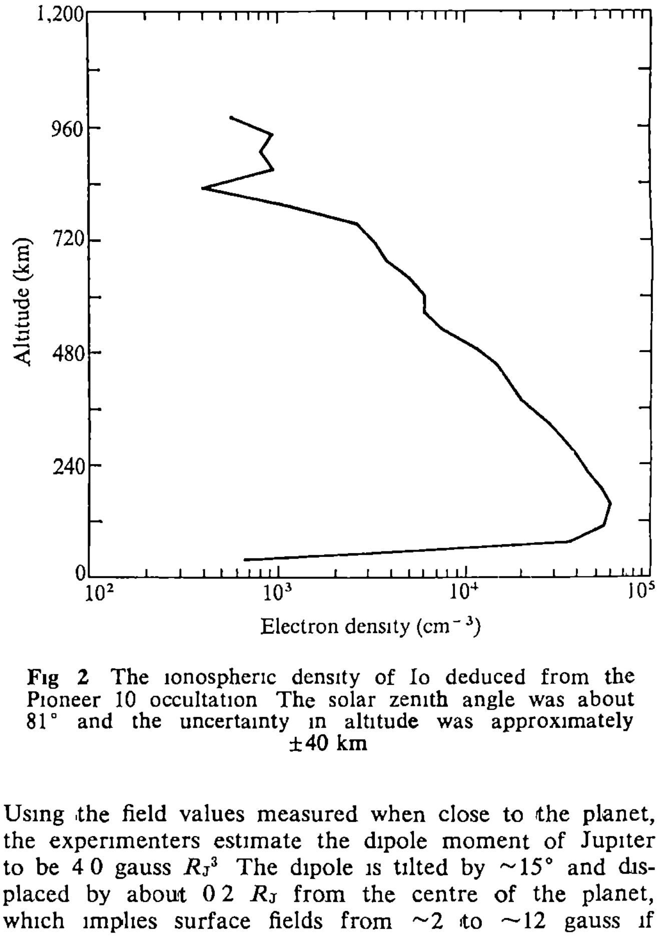

Fig 2 The ionospheric density of Io deduced from the Pioneer 10 occultation The solar zenith angle was about 81° and the uncertainty in altitude was approximately ±40 km

Smith *et al* suggest that their observations could be explained by a disk-like magnetosphere, radically different from the Earth's, although they do not rule out the possibility of a thick magnetosphere The reason for such a non-spherical 'magnetodisk' may be that the density and temperature of the magnetospheric plasma are so great that the magnetic field cannot contain the plasma at distances larger than about 20 R_J Inside that distance, it would resemble the Earth's magnetosphere, and outside, plasma would be thrown out, forming a magnetodisk something like the solar wind, with a spiral magnetic field, but which would also have some of the properties of the Earth's magnetotail, such as a neutral sheet

Plasma analyser and energetic particle detectors

The plasma analyser of Wolfe *et al*[4] measured proton spectra from 100 eV to 18 keV, electron spectra from 1 to 500 eV, charged-particle fluxes from 1×10^2 to 3×10^9 $\text{cm}^{-2}\ \text{s}^{-1}$, and the plasma flow velocity Their measurements of the bow shock correlated well with the magnetometer observations They found that the electron energy tended to peak at about 3 eV within the magnetosphere The ion energies were too small to register but the authors suggest that the outer magnetosphere of Jupiter is in a high-beta regime—in other words, the thermal energy dominates the magnetic energy

A most surprising result of the plasma analyser measurements was that the spacecraft apparently passed through the magnetosheath twice while approaching Jupiter, as if there were not one but two magnetospheric bow shocks The first occurred at about 100 R_J, and the second at about 50 R_J As the spacecraft left Jupiter three more shocks were observed Wolfe *et al* suspect that the solar-wind pressure may have fluctuated, causing the bow-shock distance to vary, but it has also been suggested that this could be an effect of the eccentricity and tilt of the Jovian magnetic dipole One suspects that if the plasma is ejected into the magnetodisk from near the magnetic equator, the plasma may create giant waves in the magnetodisk, so the spacecraft may have passed through several troughs and crests of a wavy magnetodisk

The flux of relativistic ($\gtrsim 3$ MeV) electrons, of protons above 0 5 MeV, and of 1 MeV per nucleon helium nuclei were measured by Simpson *et al*[6] with the charged-particle detector, and electrons above 70 keV were observed by Van Allen *et al*[7] with the Geiger-tube telescope The cosmic-ray telescope results reported by Trainor *et al*[8] (F B McDonald, principal investigator) provided data on high-energy electrons, protons, and helium nuclei, including high-intensity flux measurements of protons from 0 5 to 21 MeV and electrons from 0 1 to 1 MeV The trapped radiation detector of Fillius and McIlwain[9] measured fluxes, energy spectra, and angular distributions of 100 keV to 50 MeV electrons and of protons above 70 MeV

One month before encounter, the Simpson group detected bursts two days long of electrons up to 30 MeV in energy, analogous to precursors of the terrestrial magnetosphere but far more energetic The flux of relativistic electrons[6] reached $\sim 5 \times 10^8$ $\text{cm}^{-2}\ \text{s}^{-1}$ near closest approach (2 8 R_J), which was about an order of magnitude greater than the upper limit of the Jupiter Radiation Belt Workshop[31] The Workshop electron model was based to a large extent on terrestrial observations of the radio emissions of Jovian relativistic electrons, and the weaker magnetic field found by Jupiter accounts in part for the underestimate of the electron flux For protons, there were no such observational data prior to Pioneer, yet the observed[6] peak flux of $\sim 4 \times 10^6$ protons $\text{cm}^{-2}\ \text{s}^{-1}$ was near the Workshop nominal prediction The proton flux falls off within $\sim 3\ 5$ R_J, however, which was unexpected

All four groups found the particles to be highly concentrated near the magnetic equatorial plane from ~20 to ~100 R_J, and remarkably different from the terrestrial magnetosphere On the outward journey through the magnetodisk, bursts of energetic electrons and protons were found with time scales of minutes, which had great directional anisotropies[8] The experimenters noted similarities with phenomena of the Earth's magnetotail, which are understandable if the Jovian magnetic field is confined to a disk outside ~20 R_J, since one would expect the field to be stretched out in the disk in a manner similar to a magnetotail They found that the particles are trapped in a manner similar to the Earth's magnetosphere only for distances within about 20 R_J

Mead and Hess[32] had proposed that the Galilean satellites could sweep up some of the energetic particles, and Pioneer fluxes did indeed dip while passing the orbits of Europa, Io, and possibly Ganymede, but not that of the most distant and theoretically least influential, Callisto[7-9] Perhaps the proton decrease within 3 5 R_J is related to sweeping-up by the small satellite Amalthea at 2 5 R_J, which was also suggested by Mead and Hess[6 32]

Asteroid and meteoroid detectors

Two instruments on Pioneer detected micrometeoroid and larger particles The photomultiplier telescopes of Soberman et al[13] detected particles ranging from 35 μm to 10 cm at distances from 2 to 3 5 AU The pressure cells of Kinard *et al*[14] detected particles of mass $\sim 10^{-9}$ g, and showed that the asteroid belt had no more of these particles than the regions before and after the belt As was expected, the number of particle detections by the latter instrument rose dramatically when it arrived at Jupiter Kinard *et al* do not yet know whether the particles are interplanetary ones attracted by Jupiter, or particles actually orbiting the planet

Trajectory analysis and spacecraft survival

Anderson *et al*[15] have studied the Doppler shifts of the S-band transmissions of Pioneer 10 to determine the spacecraft trajectory with great precision They expect to be able to measure the masses of the Galilean satellites to 1% by studying the perturbations they cause on the trajectory They have already found that Io is about 20% heavier than previously thought, making its density 3 5 g cm^{-3}, slightly denser than our own Moon, which places an important constraint on chemical models of its interior If the pre-Pioneer densities of the other Galilean satellites[21] are approximately correct, this new Io density implies that the density of Galileans decreases with distance from the planet, analogous to the general behaviour of planet densities with distance from the Sun, which is a clue to the origin and evolution of the Solar System

Anderson *et al* also expect to measure several of the higher harmonics of the Jovian gravitational field, giving information on the distribution of mass within Jupiter obtainable in no other way This should tell us something about whether Jupiter has a core, and what its composition is, and whether there is an internal mass-asymmetry related to the eccentric magnetic field

An important datum provided by Pioneer 10 was that it survived passage through the asteroid belt and the Jovian magnetosphere It received an integrated dose of 2×10^5 rads from electrons and 5×10^4 rads from protons greater than 30 MeV, where 500 rads is a lethal human dose[17] This was very nearly the maximum dose that the spacecraft could withstand In addition to that, it is known that a spacecraft in a Van Allen belt may charge up to potentials large enough to damage the instruments[33], and it has been suggested that this charging phenomenon could be a danger to Pioneer (N M Brice, private communication, and unpublished work of F L Scarf and R W Fredericks) The fact that the spacecraft survived this danger is consistent with the presence of an unexpectedly large density of magnetospheric plasma, whose presence would tend to nullify the charging caused by energetic particles Fillius and McIlwain[9], however, do not rule out the possibility of serious spacecraft charging, which, they point out, may bias charged-particle measurements

The future

Pioneer 10 has confirmed as inescapable the fact that Jupiter radiates more energy than it receives from the Sun The source of this radiation is a major theoretical problem Models of the Jovian interior must not only explain this, but must also explain the eccentricity of the magnetic field and the values of the gravitational parameters of the planet The observed He/H abundance will serve as a constraint on atmospheric and interior models

Jovian meteorology is much more complex than had been supposed, and new atmospheric models must be constructed, probably incorporating dust The multitude of theories of the Great Red Spot may at last be tested against the new data

It is up to theoreticians to understand why the magnetosphere of Jupiter proved to be so utterly different from what we had expected At this early date, it seems to be due to unexpectedly large amounts of magnetospheric plasma, so it may be that the rate of plasma input to the magnetosphere is much larger than has been estimated or that the storage time is much greater

The distribution of energetic electron and proton fluxes can now be tested against existing theories of particle-diffusion mechanisms (refs 34–36 and unpublished work of S A Jacques and L Davis jun) Interpretation of the decimetric radiation has been complicated by the fact that the synchrotron emission is a function of both the electron energy spectrum and the magnetic field Now that we know the strength and structure of the magnetic field, we can use the theory of synchrotron radiation to extract the relativistic electron energy spectra from Earth based radio-telescope observations of the decimetric noise If the deduced energy spectra are consistent with the Pioneer measurements, this will verify the calibration of both the energetic electron sensors and the magnetometer We can then use past and future earthbound observations of the decimetric noise to measure the relativistic electron energy spectra as a function of time and position in the Jovian Van Allen belts

Io is now an even more intriguing object than before It has an atmosphere, an ionosphere, a torus, sodium atoms, brightness fluctuations, possible auroras, and it appears to control the decametric noise bursts Theories of the interaction of Io with the Jovian magnetic field, designed to explain the radio bursts, must now incorporate a conducting ionosphere It has even been suggested that Io may be the source of the Van Allen belt electrons[18]

Pioneer 10 is not only the first spacecraft to reach Jupiter, it will be the first to leave the Solar System It carries on it the famous plaque bearing a drawing of a man and woman, and telling, in the language of mathematical physics, the location of its home planet[37]

As Pioneer 10 leaves the Solar System, its near-twin, Pioneer 11, is in the asteroid belt, and is scheduled to arrive at Jupiter next December At present, NASA has decided to give it a trajectory which would take it to Saturn in 1979, but which might expose it to dangerous radiation levels at Jupiter[38] Two Mariner spacecraft are scheduled to be launched in 1977 to visit Jupiter and Saturn

Many of the discoveries of Pioneer 10 can be economically described by the statement that Jupiter resembles the Sun more than the Earth In composition, energy production, radio noise, differential rotation, field eccentricity, and the interaction of its plasma with its magnetic field, Jupiter displays truly stellar qualities

This paper is dedicated to the memory of Professor Neil M Brice, whose recent tragic death withdrew from the world a brilliant scientist, a wonderful human being, and a very good friend Whatever growth as a scientist I may have attained in the last few years, I owe largely to his encouragement, stimulation, enthusiasm, energy and constructive criticism

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Trapped xenon in meteorites

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Xenon in meteorites can be resolved into a mixture of component X and trapped xenon with the following composition, ^{124}Xe ^{126}Xe ^{128}Xe ^{130}Xe ^{131}Xe ^{132}Xe ^{134}Xe ^{136}Xe $= 0.0276$ 0.0248 0.501 1.00 5.04 6.19 2.31 1.90 *This trapped meteoritic xenon is distinct from xenon trapped in the average carbonaceous chondrite which is shown to represent the average composition of the total xenon in meteorites containing various mixtures of component X and trapped meteoritic xenon*

IN 1960, Reynolds[1] reported the discovery of radiogenic ^{129}Xe, the decay product of extinct ^{129}I, in the Richardton chondrite. He also noted a general anomaly pattern across the isotopes of xenon in the Richardton meteorite, and later that year he reported[2] a similar isotopic anomaly pattern in xenon released from the Murray carbonaceous chondite. Since Murray contained over 40 times more xenon than Richardton, but less cosmogenic noble gas isotopes than Richardton, Reynolds[2] concluded that the general anomaly pattern was characteristic of primordial xenon.

Following Reynolds[1,2] discovery of general isotopic anomalies in meteoritic xenon, there were other attempts to determine accurately the isotopic composition of xenon trapped in meteorites[3–5]. Apart from a component similar to atmospheric xenon which is released at low extraction temperatures and is presumably due to contamination of meteorite surfaces by the atmosphere, these studies[1–5] showed that the xenon trapped in average carbonaceous chondrites (AVCC Xe) may be a mixture of fractionated atmospheric-type xenon plus a fission component. But, the concept of AVCC Xe as primordial, when defined as a mixture of two components, was unclear, and this concept has become increasingly vague as the results of stepwise heating experiments on different carbonaceous chondrites in several laboratories[6–13] demonstrate convincingly that the two components of AVCC xenon are inhomogeneously mixed.

Accurate measurements of the isotopic composition of xenon from the Sun might help elucidate the nature of primordial xenon in our Solar System, and a component of solar xenon has recently been identified in the Pesyanoe meteorite[14] and in lunar soil[15–20]. The general features of this solar xenon can be related to atmospheric xenon by isotopic mass fractionation, although certain xenon isotopes consistently deviate from the general fractionation pattern[15–22]. It has been suggested that deviations from the general isotopic fractionation pattern are due to radiogenic ^{129}Xe in the atmosphere[18,22], to excess ^{131}Xe and ^{132}Xe in the atmosphere[18], to fissiogenic ^{134}Xe and ^{136}Xe in lunar fines[16–18,20–22], and to either an excess of ^{126}Xe, or a deficiency of ^{124}Xe, in solar xenon as a result of nuclear reactions[20,23]. Eberhardt *et al.*[16] have also suggested the possibility that fractionation might not be responsible for major differences in the isotopic composition of solar and terrestrial xenon, but that these two types of xenon might originate from different nucleosyntheses.

We have noted[24] that the xenon released from carbonaceous chondrites at ≈600°–1,000° C contains an excess of the proton-rich isotopes, $^{124-128}$Xe, and the neutron-rich isotopes, $^{131-136}$Xe. This observation suggests that meteoritic xenon consists of two isotopically distinct components whose presence cannot be explained by nuclear or fractionation processes within the meteorites. The meteoritic xenon component which is enriched in both the light and the heavy isotopes relative to ^{130}Xe was referred to as X, but the nature of the other meteoritic xenon component was not clearly defined. In this report the available data on xenon in carbonaceous chondrites, in lunar fines, and in the Earth's atmosphere are examined to seek a better understanding of the nature of these two components of meteoritic xenon.

General isotopic anomaly patterns of xenon

The isotopic composition of xenon in the three major reservoirs of xenon which have been studied, the Earth's atmosphere, meteorites, and lunar soil is presented in Table 1. The isotope ^{129}Xe is excluded because it is produced[1] in the early Solar System by the decay of primordial ^{129}I.

In Table 1, the nonradiogenic and nonspallogenic isotopes of xenon in lunar fines are used to represent the composition of solar xenon. The results from various laboratories are shown because estimates on the composition of xenon in lunar fines differ significantly between them, and there are differing opinions on the actual isotopic composition of xenon in the solar wind. These various estimates of solar xenon do however show a systematic relationship which suggests that they may be related by mass-dependent fractionation[20].

Two estimates for the composition of xenon in meteorites are represented in Table 1 by AVCC and WAVCC. The values for AVCC are an average of the estimate of trapped meteoritic xenon based on seven meteorites analysed by Marti[4] and three

Table 1 Isotopic composition of solar, meteoritic and atmospheric xenon

Sample	^{124}Xe	^{126}Xe	^{128}Xe	^{130}Xe	^{131}Xe	^{132}Xe	^{134}Xe	^{136}Xe	Refs
Solar									
Lunar 16	2.99	2.78	51.0	100	494.7	600.6	220.1	176.0	19
Lunar Fines—10084 59	3.02	2.93	51.5	100	498.0	606.0	220.0	179.0	15
Lunar Fines—10084 48	2.94	2.55	50.8	100	496.0	608.0	224.2	182.6	17
Lunar Fines—SUCOR	2.89	2.63	50.9	100	499.0	606.6	225.2	182.7	18
Lunar Fines—10084 47	2.99	2.67	50.45	100	495.5	607.0	225.8	184.2	16
Lunar Fines—15601 64	2.73	2.61	50.6	100	501.2	613.5	229.4	187.1	20
Meteoritic									
AVCC	2.828	2.533	50.38	100	506.7	620.9	236.9	199.0	4, 5
WAVCC	—	—	50.82	100	506.3	617.9	235.5	197.2	This work
Atmospheric									
Air	2.353	2.206	47.03	100	519.1	659.1	255.9	217.4	25
Air	2.334	2.175	47.07	100	522.4	661.4	256.7	218.2	18

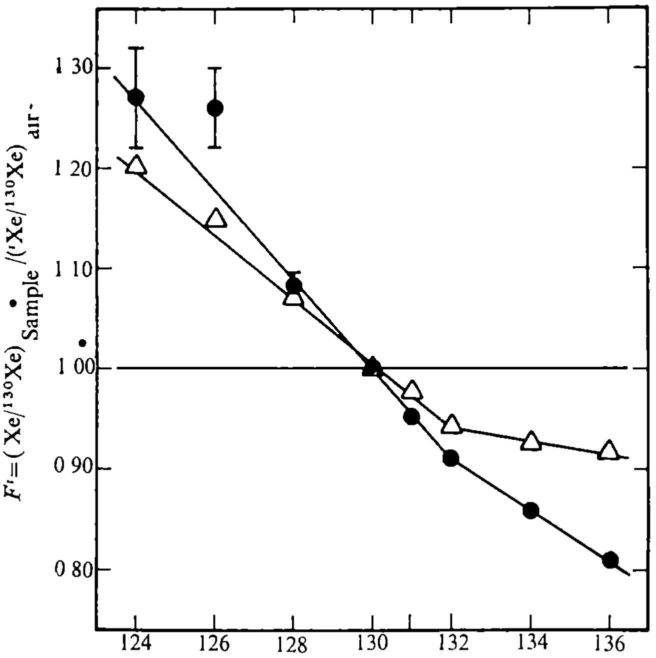

Fig 1 A comparison of AVCC xenon[4,5] (△) and solar xenon[19] (●) with xenon in the Earth's atmosphere[25]. The break in the lines at ^{132}Xe indicates that neither of these two forms of extraterrestrial xenon can be related to atmospheric xenon by mass-dependent fractionation.

carbonaceous chondrites analysed by Eugster *et al*[5]. The values shown for WAVCC represent a weighted average for approximately 75 analyses in different laboratories on a total of 35 carbonaceous and gas-rich meteorites, as listed in Table 5 of Mazor *et al*[26]. These results were weighted according to the xenon content of the meteorites. The abundances of the two light xenon isotopes, ^{124}Xe and ^{126}Xe, are not shown in WAVCC as there are no data on these isotopes for many of the analyses[26]. The lower abundance of the heavy xenon isotopes in WAVCC than in AVCC indicates that the relative abundance of the heavy isotopes is lower in xenon-rich meteorites than in average carbonaceous chondrites. Atmospheric xenon is represented in Table 1 by the widely quoted analysis by Nier[25] and by a recent analysis by Podosek *et al*[18].

To compare the composition of xenon in these three reservoirs, we used the method of Canalas *et al*[27], in which the abundance of each isotope of mass number, i, is compared with its atmospheric abundance by the factor, F^i, defined as follows

$$F^i = ({}^{i}\mathrm{Xe}/{}^{130}\mathrm{Xe})_{\mathrm{sample}}/({}^{i}\mathrm{Xe}/{}^{130}\mathrm{Xe})_{\mathrm{air}} \qquad (1)$$

The values of F^i for solar and AVCC xenon are plotted against mass number, i, in Fig 1. For this comparison we have used Nier's value for atmospheric xenon[25] and Kaiser's value for solar xenon[19]. The latter was selected because it represents an extreme of the estimates for solar xenon and has been earlier depicted as solar xenon with the lowest fission component.

Several investigators[14-17] have suggested that solar xenon and AVCC xenon are identical, except for the presence of excess $^{132-136}$Xe in the latter. Atmospheric xenon and AVCC xenon can be related by mass-dependent fractionation[1-5], except for the presence of excess $^{132-136}$Xe in the latter. Thus, the view of solar and AVCC xenon expressed by these investigators[14-17] suggests that atmospheric and solar xenon are directly related by fractionation. Kaiser[19] has recently interpreted his results on xenon in Luna 16 as evidence that atmospheric xenon is, in fact, fractionated solar xenon. But, as can be seen in Fig 1, even for the extreme estimate of solar xenon as given by Kaiser[19], there is steeper fractionation across the light isotopes, $^{124-131}$Xe, than across the heavy isotopes, $^{132-136}$Xe. The break in the fractionation patterns for both AVCC and solar xenon at ^{132}Xe clearly shows that neither of these two reservoirs of extraterrestrial xenon can be related to atmospheric xenon by simple mass-dependent fractionation.

Component X and trapped meteoritic xenon

Reynolds and Turner[6] have reported the presence of excess heavy xenon isotopes in the gas released from the Renazzo carbonaceous chondrite at 700°–1,000° C. Stepwise heating experiments on other carbonaceous chondrites in several laboratories[7-13] confirmed this anomaly. We have reported[24] that the results of stepwise heating experiments showed an enrichment of ^{124}Xe in the same temperature fractions which contained excess ^{136}Xe. The correlation observed between light and heavy isotopes could not be explained by any of the previously proposed mechanisms, and we concluded that the observed anomaly pattern was caused by the release of an isotopically distinct component of xenon, referred to as component X, which had been incorporated into the meteorites.

Phinney's[13] analyses for xenon released by stepwise heating of carbonaceous chondrites for the temperature fractions showing the largest enrichment of ^{136}Xe are listed in Table 2 together with the results of stepwise heating experiments discussed in our earlier report[24].

The correlation between the enrichment of the light isotopes, ^{124}Xe and ^{126}Xe, and the enrichment of the heaviest isotope, ^{136}Xe, in the xenon released by stepwise heating can be seen in Fig 2, where ^{124}Xe/^{130}Xe and ^{126}Xe/^{130}Xe are plotted against ^{136}Xe/^{130}Xe. Estimates of solar, atmospheric, and meteoritic xenon from Table 1 are shown for comparison. As can be seen from Fig 2, the data of Phinney[13] agree with the correlation reported earlier[24]. The abundances of ^{124}Xe and ^{126}Xe have not been corrected for cosmic-ray induced spallation reactions, which may partially account for the scatter of data points from the correlation lines.

The dashed line in Fig 2 shows the relationship expected from diffusive fractionation[28], and the failure of solar xenon to lie on the fractionation line passing through atmospheric xenon is understood in terms of the relationship shown in Fig 1. The solid line connecting atmospheric and solar xenon defines the isotopic composition of xenon which would be produced by mixing components from these two xenon reservoirs. This 'mixing' line approximately parallels the fractionation relationship. If AVCC Xe really represents an average of the total xenon in meteorites, then the composition of trapped meteoritic xenon, when corrected for the presence of component X, would be

Table 2 Anomalous xenon released in stepwise heating of the carbonaceous chondrites

Sample	^{124}Xe	^{126}Xe	^{128}Xe	^{130}Xe	^{131}Xe	^{132}Xe	^{134}Xe	^{136}Xe	Refs
Renazzo 800° C	2.93±0.05	2.57±0.03	50.6±0.4	100	505.8±2.4	617.2±1.9	243.3±2.2	210.4±1.5	6
Leoville 1,010° C	3.07±0.09	2.66±0.12	51.4±0.5	100	511.3±2.4	625.0±3.9	250.0±2.4	220.6±2.3	11
Mokoia 700° C	3.43±0.05	3.02±0.06	51.9±0.4	100	512.0±2.5	625.0±2.4	269.7±1.3	244.8±1.2	13
Mokoia (matrix) 600° C	3.15±0.09	2.72±0.09	52.7±0.7	100	519.9±6.8	633.3±7.6	276.1±3.6	248.9±3.6	9
Mokoia 750° C	3.39±0.06	2.90±0.05	52.0±0.8	100	514.1±5.6	624.5±5.6	278.6±3.2	261.1±2.0	12
Mokoia 800° C	3.44±0.04	2.83±0.04	52.2±0.2	100	514.8±1.8	628.9±1.9	280.8±1.0	263.6±1.1	13
Allende 700° C	3.37±0.10	2.93±0.10	50.7±0.5	100	516.3±3.2	633.7±3.5	289.2±2.7	273.6±3.0	13
Allende 1,000° C	3.39±0.14	2.77±0.09	54.5±0.9	100	513.0±6.1	624.9±8.6	291.2±3.9	282.5±2.4	12
Allende 600° C	3.42±0.09	3.02±0.12	52.1±0.9	100	522.7±5.6	643.1±6.2	300.8±5.0	290.0±3.4	13
Allende 800° C	3.62±0.13	2.84±0.09	52.1±1.5	100	518.6±11.9	641.0±14.3	305.1±7.0	299.4±5.4	12

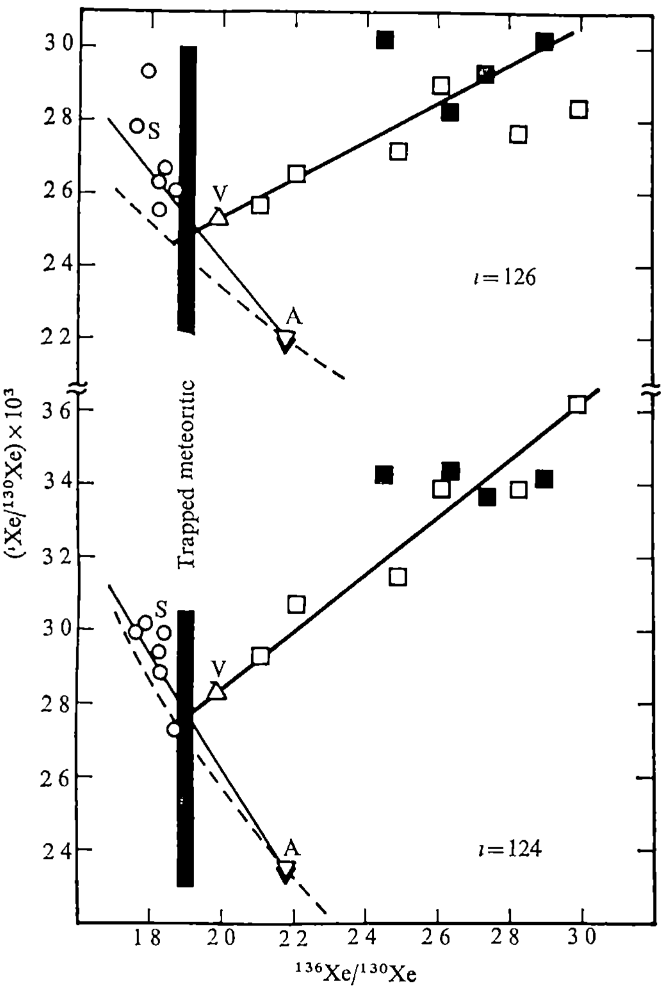

Fig 2 The correlations of $^{124}Xe/^{130}Xe$ and $^{126}Xe/^{130}Xe$ with $^{136}Xe/^{130}Xe$ in the xenon released by stepwise heating of carbonaceous chondrites The correlations between these xenon isotope ratios are shown by the heavy lines ■ Represents recent stepwise heating data by Phinney[13] The symbols S, V, A are shown above solar, AVCC, and atmospheric xenon, respectively The dashed line passing through atmospheric xenon shows the isotope compositions expected from diffusive fractionation, and the solid line passing through atmospheric and solar xenon show the isotopic compositions expected from mixing these two types of xenon

expected to lie to the left of AVCC Xe on the correlation lines shown in Fig 2 Likewise, the subtraction of component X from the xenon released at each extraction temperature might leave a common trapped meteoritic xenon component Since the other two known reservoirs of xenon are solar and atmospheric, a suitable choice for trapped meteoritic xenon can be characterised by the intersections of the correlation lines with the 'mixing' line or the fractionation line The darkened column labelled trapped meteoritic in Fig 2 represents these intersections Until the isotopic composition of solar xenon is sufficiently well established to indicate clearly the relationship between solar and atmospheric xenon, a decision on which intersection represents 'trapped meteoritic' xenon would be arbitary Since these three reservoirs of xenon are probably genetically related, though not necessarily by a diffusive fractionation relationship, we will postulate a simple linear relationship between atmospheric and solar xenon to see if meteoritic xenon can be resolved into a mixture of component X and a self-consistent composition for trapped meteoritic xenon

The observed correlations between the other nonradiogenic xenon isotopes released in the stepwise heating of carbonaceous chondrites are shown in Fig 3, together with the 'mixing' line between solar and atmospheric xenon All the xenon isotope data from Table 1 and Table 2 for i = 128–136 are shown in Fig 3, except for the ratio, $^{128}Xe/^{130}Xe$, in the xenon released from the Allende meteorite at 1,000° C The unusually high value for this ratio, indicated by the arrow in Fig 3, is attributed to neutron-capture reactions on ^{127}I in this meteorite Firemen *et al*[29] and Manuel *et al*[12] have reported an excess of certain noble gas isotopes in Allende as a result of neutron-capture reactions on halogens

From Fig 3 it appears that the points of intersection of the correlation lines and the 'mixing' lines define a self-consistent composition for trapped meteoritic xenon, and this composition is also consistent with the trapped xenon shown in Fig 2 The isotopic composition of trapped meteoritic xenon defined by these intersections is ^{124}Xe ^{126}Xe ^{128}Xe ^{130}Xe ^{131}Xe ^{132}Xe ^{134}Xe ^{136}Xe = 0 0276 0 0248 0 501 1 00 5 04 6 19 2 31 1 90 The scatter of data points about the correlation lines in Fig 3 may be due to variations in the compositions of trapped meteoritic xenon If the trapped meteoritic xenon lies along the 'mixing' line, as would be expected if meteoritic xenon has been acted on by the same process responsible for the relationship between atmospheric and solar xenon, then this might account for the smallest scatter of data points along the correlation line for i = 134 For this isotope, the slope of the 'mixing' line and the slope of the correlation line are most nearly equal

It should be noted from Fig 3 that, relative to ^{130}Xe, all the other xenon isotopes are enriched in component X when compared with their abundance in trapped meteoritic xenon We have considered[24] possible origins for component X earlier and shall now discuss the relationship between AVCC xenon, trapped meteoritic xenon, and component X

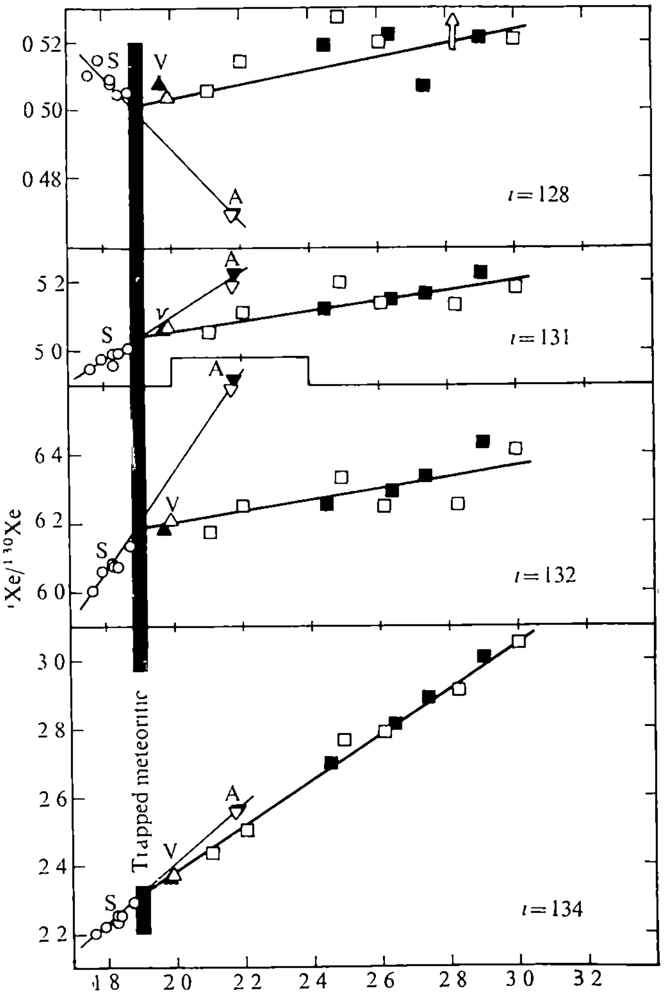

Fig 3 The correlation of other xenon isotope ratios with $^{136}Xe/^{130}Xe$ in the xenon released by stepwise heating of carbonaceous chondrites The lines and symbols are as defined for Fig 2 except that both AVCC and WAVCC are shown below the symbol V

AVCC xenon

Mazor *et al*[26] have shown the results of approximately 75 analyses for meteoritic xenon from several laboratories These results are for total xenon and do not include data on ^{124}Xe and ^{126}Xe for most of the meteorites No data on ^{130}Xe are given for four of the analyses Thus, it is not possible to determine whether the total xenon in these meteorites shows a correlation between an enrichment of the light isotopes and an enrichment of the heavy isotopes, as shown in Fig 2 for stepwise heating experiments

But, even in total melt experiments, we find variations in the relative abundances of the heavy xenon isotopes as observed for stepwise heating experiments This correlation of excess ^{136}Xe with excess ^{134}Xe in about 70 total melt analyses of the xenon in 35 different meteorites is shown in Fig 4 The dashed lines indicate the area of uncertainty for the correlation which would result if points on the solid correlation line had an uncertainty of $\pm 1\%$ This degree of uncertainty is typical of the xenon isotope measurements, and this is shown for a symbolic data point in the lower right of Fig 2 The isotopic compositions given in Table 1 for AVCC and WAVCC are shown in Fig 4 The fact that these lie near the middle of the graph, which is heavily populated with data points representing total meteoritic xenon, demonstrates that AVCC and WAVCC satisfactorily describe the average isotopic abundances of ^{130}Xe, ^{134}Xe and ^{136}Xe in total meteoritic xenon

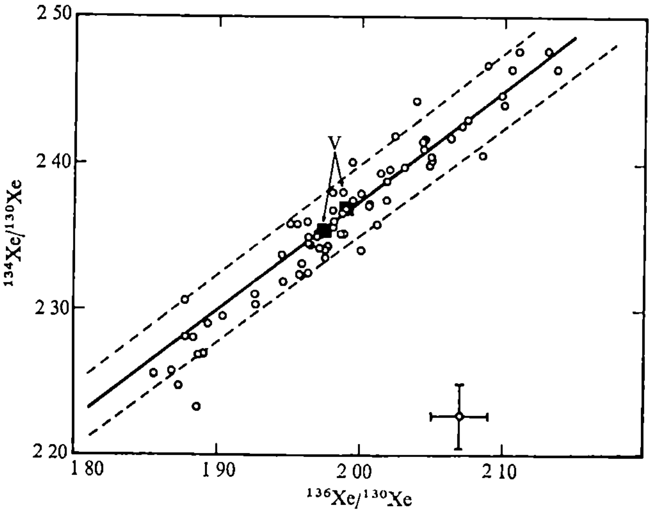

Fig 4 The correlation of ^{134}Xe/^{130}Xe with ^{136}Xe/^{130}Xe in the total xenon of meteorites Data are from a compilation by Mazor *et al*[26] The darkened squares, labelled, V, show the isotopic composition of AVCC and WAVCC xenon Typical error limits of $\pm 1\%$ are shown in the lower right corner

The correlation of excess ^{134}Xe with excess ^{136}Xe for total meteoritic xenon, in the manner observed for xenon released by stepwise heating experiments, suggests that the same mechanism may account for both the isotopic variations in different temperature fractions of a meteorite and the isotopic variations in the total xenon from different meteorites Recently Takaoka[30] reiterated the view that correlations between isotope ratios for ^{128}Xe, ^{130}Xe, ^{131}Xe, ^{132}Xe, ^{134}Xe and ^{136}Xe in the total xenon of different carbonaceous chondrites can be accounted for by the carbonaceous chondrite fission (CCF) hypothesis[6] Takaoka did not, however, consider the isotopic abundances of ^{124}Xe and ^{126}Xe Although the correlation shown in Fig 4 is consistent with both the CCF hypothesis and the component X hypothesis, we assume that the correlation results from variations in the relative amounts of component X for the following reason This same mechanism can also account for isotopic correlations observed in stepwise heating experiments, whereas the CCF hypothesis fails to account for the correlations of excess ^{124}Xe and excess ^{126}Xe with excess ^{136}Xe, as shown in Fig 2

Another mechanism which may contribute to differences in the isotopic composition of atmospheric, solar and AVCC xenon has been noted by Kuroda[31] He suggests that neutron-capture reactions in the interior of the sun during the deuterium-burning stage may have enriched solar xenon in the intermediate mass isotopes, $^{128-132}$Xe Xenon which had been isolated from the sun before the onset of deuterium burning would thus appear to be enriched in the lighter and heavier isotopes, a pattern observed in component X Fractional differences between the abundances of ^{130}Xe in component X and other sources are, however, much larger than those which Kuroda[31] attributes to solar nuclear reactions

From the range of ^{136}Xe/^{130}Xe values shown in Figs 3 and 4 we conclude that for total melt experiments the ^{136}Xe/^{130}Xe ratio may differ from the ^{136}Xe/^{130}Xe ratio in trapped meteoritic xenon by $\approx$0–11% as a result of variations in the relative amounts of component X, whereas stepwise heating experiments partially separate these two components of meteoritic xenon to yield ^{136}Xe/^{130}Xe ratios that are $\approx$0–58% higher than the ^{136}Xe/^{130}Xe ratio in trapped meteoritic xenon AVCC xenon represents the isotopic composition for the average observed mixture of trapped meteoritic xenon and component X

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Gas streaming in 2U0900-40 and Cyg X-1

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Published photometric data on 2U0900-40 and Cyg X-1 are interpreted as evidence of gas streaming in these systems The location and extent of the gas streams indicate that they originate from the accretion disk and extend to the far side of the primary We suggest that large scale mass loss is occurring from the outer regions of the accretion disk as a result of radiative acceleration by a high luminosity component of soft X rays ($\varepsilon \lesssim 1$ *keV*)*, and offer a possible interpretation of the radio observations of Cyg X-1*

Several X-ray sources have been identified with binary systems the light curves of which show two maxima and two minima per orbital period[1–5] characteristic of 'ellipsoidal' variables The recently reported failure to detect the secondary minimum of Cyg X-1 (refs 6–7) has, however, cast some doubt on the conventional interpretation of the light curves in terms of gravity and limb darkening effects of a tidally distorted star Here we suggest a possible interpretation of both the Cyg X-1 observations, and the recently published photometric data on HD77581 which indicates variability of the light curve following secondary minimum, in terms of a model invoking gas streaming on the 'far side' of the primary as viewed from the X-ray source It is suggested that the variable radio emission observed in Cyg X-1 could originate in an expanding envelope surrounding the binary system

Evidence of gas streaming

Wickramasinghe and Whelan[8] and Hutchings[9] have attempted to analyse the light curve of HD77581 in the framework of a model in which the primary is in near contact with its inner critical Roche lobe but were unable to obtain a detailed fit near secondary minimum Agreement between theory and observations was found to be better if an eccentric orbit is assumed[9] This interpretation, we feel, can now be ruled out on the basis of the detailed observations recently published by Jones and Liller[10] which we present in Fig 1 (Phase $\varphi = 0$ corresponds to X-ray eclipse—also secondary minimum) The solid line corresponds to the theoretical model computed by Wickramasinghe and Whelan[8] assuming a circular orbit The failure to recover full brightness following X-ray eclipse on March 5, 1973, as shown by these observations is strongly suggestive of obscuration of the primary between phases $\varphi = 0$ and $\varphi = 0\ 5$ There is also some evidence for a similar effect on a smaller scale over the same phase in the previous cycle and for even smaller effects at other phases

Any outflow of matter from the primary (defined as the more massive component) through the inner Lagrangian point L_1 is likely to be subsonic and therefore cannot give rise to a gas stream which extends to the far side of the star One of us[11] has recently pointed out the possibility of radiative acceleration of material at the outer edges of the accretion disk by a high luminosity component of soft X rays (of energy $\varepsilon < 1$ keV) In this case, it is possible that some of the radiatively accelerated material could give rise to an optically thick gas stream which falls back on the surface of the primary as illustrated in Fig 2 Alternatively, the material could be accelerated to velocities in excess of the escape velocity resulting in mass loss from the system Either way the flow is expected to be concentrated in the orbital plane with the density being highest near the stellar surface The observed anisotropy of the flow with respect to the line of centres is due to the initial angular momentum of the accelerated material We note that in addition to the diminution in light, the (U–B) and (B–V) colours are expected to become bluer during the equatorial obscuration, since only the hotter polar regions will be visible There is some evidence for this effect in the observational data (Fig 1)

An optically thick region of thickness $\Delta h_{st} \sim 1/10\ R_s$ (normal to the orbital plane) where $R_s \sim 30\ R_\odot$ is the stellar radius, would suffice to explain the observed (0 08 mag) diminution in light at phase $\varphi \approx 0\ 25$ Adopting a value of $5\ R_\odot$ for the radial thickness of the effective obscuring region, we estimate an electron number density $n_e \gtrsim 10^{13}$ cm^{-3} assuming that electron scattering is the dominant opacity source A lower density may be appropriate if the observed extinction occurs over an extended region in the orbital plane

A lower limit to the rate of mass transfer in the gas stream is given by $m_H \Delta h_{st}\ V_g/\sigma_T$ where σ_T, m_H and V_g are the electron scattering cross section, the mass of the hydrogen atom and the stream velocity respectively Assuming that V_g is near the escape velocity the above expression yields $\sim 10^{-7}\ M_\odot$ yr^{-1} The rate of mass loss from the entire disk is thus likely to be at least $10^{-6}\ M_\odot$ yr^{-1} and could be considerably greater

Such a high rate of mass ejection from the accretion disk of the secondary apparently occurs only during some cycles although there is probably continuous outflow at much lower densities This, together with the fact that the observed X-ray luminosity ($\sim 10^{37}$ erg s^{-1}) requires a steady accretion rate of 10^{-8}—$10^{-9}\ M_\odot$ yr^{-1} (ref 12) for a neutron star or black hole secondary, suggests that a local enhancement in the rate of mass transfer from the primary through L_1 could trigger an increase in the rate of radiation-pressure driven mass loss from the outer edges of the disk with little change in the X-ray luminosity In this connection it should be noted that there is considerable observational and theoretical evidence, which supports the hypothesis that mass transfer through L_1 is the dominant mechanism of mass exchange in X-ray binaries with a supergiant component[11]

We note that the theoretical light curve near secondary

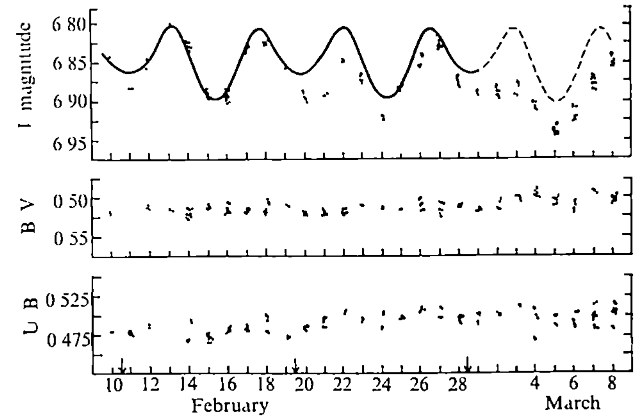

Fig. 1 The V, (B–V) and (U–B) observations of Jones and Liller[10] The arrow corresponds to X-ray eclipse The continuous curve is the zero eccentricity model of Wickramasinghe and Whelan[8] for an orbital inclination $i \sim 90°$ and a mass ratio $q \sim 0\ 09$

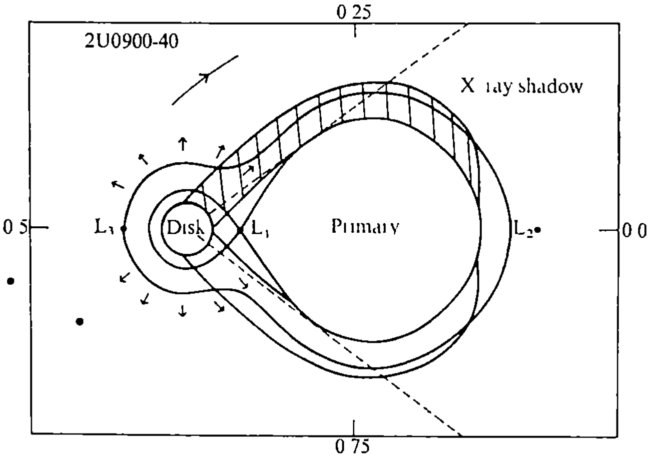

Fig 2 A schematic representation of a possible flow pattern in the orbital plane (neglecting rotation) for a mass ratio $q \sim 0.09$ The Roche equipotentials through the Lagrangian points L_1 and L_3 have been drawn to scale The hatched area represents the probable location of the gas streams causing maximum obscuration

minimum is shallower than the observed light curve even during the first cycle It is plausible that the continuous low density outflow from the disk envisaged earlier could give rise to obscuration mainly near secondary minimum when the material in the region of the X-ray shadow lies between the observer and the primary[8] The possibility of detecting the gas streams spectroscopically should also be greatest at these phases On this interpretation the gas streams nearest the stellar surface are required to fall back on the far side of the primary rather than be accelerated to velocities in excess of the escape velocity

The possibility that radiative acceleration by soft X rays may prevent the development of an appreciable stellar wind from the stellar surface facing the X-ray source has recently been discussed by one of us[11] It is accordingly interesting that the estimated mass flux in the stream indicates that rates of mass loss in excess of normal stellar wind values may be possible from the accretion disk during some cycles The flow patterns in the two cases are however likely to be quite distinct as indicated earlier A wind from the accretion disk would be concentrated largely in the orbital plane and will tend to avoid the region of the X-ray shadow

Walker[6] has observed the light curve of HD226868 over several cycles during the period May to June, 1973 and reports regular primary minima with variable secondary minima The secondary minimum is reported to be completely filled in during some cycles This result has also been confirmed by Martynov[7]

An explanation similar to that offered for HD77581 is possible if it is noted that this system is viewed at a high orbital inclination as is evident from the absence of an X-ray occultation A sufficiently extensive scattering region in the orbital plane at the far side of the primary could thus act as an additional source of light at secondary minimum

We note that the mass ratio $q(=M_2/M_1) \sim 0.6$ of the Cyg X-1–HD226868 system[13] is considerably larger than that of the 2U0900-40–HD77581 system[8] (~ 0.09) where M_1 and M_2 are the masses of the primary and secondary respectively The angular momentum problem is accordingly not as severe, and provided the initial acceleration of disk material is possible (for example by radiation pressure) gas streams may interact at the far side of the primary and form an accretion shock front which could serve as an effective scattering region (Fig 3) The exceptionally large scatter in the light curve[5] indicates that gas streams may contribute to the light at all phases

It is evident that the volume of gas responsible for the filling in of the secondary minimum must be situated sufficiently close to the primary so that it may be eclipsed at the appropriate phases (~ 0.5) A mean distance of $\sim 1.5 R_s$ from the centre of the primary may thus be appropriate (We note that the orbital inclination i must be $<< 90°$ for the present model to be viable suggesting that values of i corresponding to grazing eclipse of the secondary may be required) An order of magnitude estimate of the total number of electrons required to fill in the secondary minimum (of depth ~ 0.06 min) can be obtained from the equation $\sigma_T N_e W/\pi R_s^2 \sim 0.06$ where W is the geometrical dilution factor Setting $W \sim 1/10$, $R_s \sim 10^{12}$ cm we obtain $N_e \sim 10^{48-49}$ Assuming further that the gas occupies a volume 10^{36} cm^3 (1/10 stellar volume)—a plausible value from the eclipse point of view—we obtain an electron number density $n_e \sim 10^{12-13}$ cm^{-3} The scattering region is thus likely to be optically thin ($\tau_{es} \lesssim 1$) and could give rise to a detectable polarisation measure suggesting a possible test of the model Further consequences of the proposed model should be noted

(a) Accretion of matter on to the far side of the primary may result in the heating up of the stellar atmosphere Assuming $M_1 \sim 20\, M_\odot$ and a terminal velocity of the order of the escape velocity, we estimate a 10% increase in effective temperature for accretion rates $\sim 10^{-3}\, M_\odot\ \mathrm{yr}^{-1}$ The observed brightening of the star near secondary minimum could be the result of a general heating effect or a localised hot spot Simultaneous spectroscopic and photometric observations at the appropriate phases would help eliminate this possibility We note that on the scattering model the colours should remain almost unchanged when the secondary minimum is filled in

(b) If we assume that the scattering material is in the region of the shock, the temperature of the gas is likely to be $\sim 10^7$ K We estimate that the infrared contribution due to free–free emission should yield an excess ~ 0.5 mag in (K–M) near secondary minimum if $N_e \sim 10^{49}$ The optical contribution due to free–free emission is 0.1% of the stellar contribution and can be neglected

(c) Cyg X-1 was observed as a weak radio source (2×10^{-28} $W M^{-2}$ Hz^{-1} at 1415 MHz) in April 1971 (ref 14) The source has a nearly flat radio spectrum in the frequency range 1,415–10,630 MHz and was not detected in surveys made before this date[15] We note that free–free emission from an expanding envelope surrounding the binary system could result in an appreciable radio luminosity if the emitting region is optically thin to free–free absorption and is maintained at a high degree of ionisation The former condition requires $n_e^2 L \lesssim 2 \times 10^{25}$ (assuming a temperature $T \sim 10^4$ K characteristic of HII regions) where L is the linear dimension of the emitting region From the observed radio flux at 1,415 MHz we obtain $n_e^2 L^3 \sim 5 \times 10^{58}$ An extended region with $L \sim 5 \times 10^{16}$ cm and $n_e \sim 2 \times 10^4$ cm^{-3} would satisfy the above conditions Whether the gas can remain sufficiently ionised at distances $\sim 10^{16}$ cm from the binary system is difficult to estimate without a detailed model for the outflow.

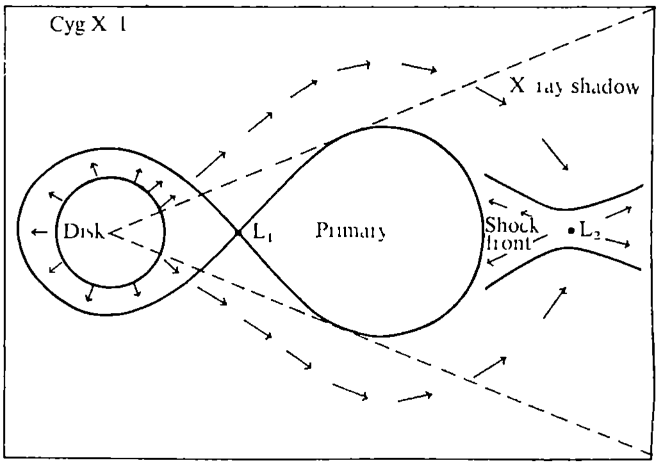

Fig 3 A schematic representation of a possible flow pattern in the orbital plane (neglecting rotation) for a mass ratio $q \sim 0.6$ The location of the accretion shock front is also indicated

We note, however, that a Stromgren sphere surrounding such a star would have a dimension ~ 10^{17} cm (ref 16) if the particle density is as high as ~ 2×10^4 cm^{-3} indicating that our suggestion is plausible

Since the details of the outflow from the accretion disk are not known it is not clear at present whether velocities in excess of the escape velocity can be achieved as a result of radiative acceleration by soft X rays Preliminary estimates indicate that continued radiative acceleration is more likely to be achieved by the stellar ultraviolet radiation Mass loss from the system may thus occur in the form of an intense 'stellar wind', the material of which originates from the accretion disk Until the details of the model are worked out, our suggested interpretation of the Cyg X-1 observations must be considered as tentative

Our conclusions may be summarised as follows

(1) The observed variations in the shape of the light curve of HD77581 following X-ray minimum can be interpreted in terms of a model invoking gas streaming The location and extent of the gas streams indicate that they originate from the accretion disk, possibly as a result of radiative acceleration The variability of the gas streams is attributed to local changes in the rate of mass transfer through the inner Lagrangian point, which in turn effects the rate of mass loss from the disk

(2) The Cyg X-1 observations can be interpreted in terms of a scattering region of volume 10^{36} cm^3 and electron density $n_e \sim 10^{12-13}$ cm^{-3} situated at a distance of ~ 1 5 stellar radii from the primary The model predicts a detectable polarisation measure and an infrared excess of ~ 0 5 mag in K–M near X-ray eclipse The observed variable radio emission could originate as a result of free–free emission from the optically thin regions of an expanding envelope surrounding the binary system

(3) In view of the possible importance of gas streaming in 2U0900-40 and Cyg X-1, the determination of orbital eccentricities from the light curves must be viewed with caution The interpretation of the radial velocity observations of these systems could also be complicated by gas streaming effects at certain phases

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DNA polymerase activity from two temperature-sensitive mutants of Rous sarcoma virus is thermolabile

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Temperature sensitivity of growth and transformation by certain early Rous sarcoma virus mutants correlates with thermolability of their virion-associated DNA polymerase The enzyme is therefore encoded by viral RNA and is required for provirus synthesis

Two temperature sensitive mutants of Rous sarcoma virus (RSV) have been isolated which have defects very early in their growth cycle (ref 1 and W S M, R R Friis, M Linial and P K Vogt, in preparation) They are called *ts*335 and *ts*337 Their temperature sensitive function is necessary for the initiation but not the maintenance of both viral replication and cell transformation (ref 2 and in preparation) The DNA polymerase activity in the mutant virions appears to be temperature sensitive when assayed with either endogenous or exogenous templates (ref 2 and in preparation) (According to the system for virus nomenclature proposed by Vogt, Weiss and Hanafusa[3] these viruses are designated as *ts*LA335PR-C and *ts*LA337PR-C)

We have purified the DNA polymerase from both *ts*335 and *ts*337 and compared the activities of the purified enzymes with those of the DNA polymerase of wild type RSV The three known activities of the avian RNA tumour virus DNA polymerase have been studied RNA-directed DNA synthesis, DNA-directed DNA synthesis, and ribonuclease H (which selectively degrades the RNA portion of a DNA-RN hybrid[4])

Thermolabile enzymes

DNA polymerase activity of detergent-treated *ts*335 and wild type RSV virions at 34°, 37° and 40 5° C was measured both in the endogenous reaction and after addition of an exogenous template primer The endogenous activity of *ts*335 was slightly lower at 40 5° C than at 34° C, while the activity of the RSV wild type increased two-fold through this temperature range (Table 1) With poly(C) oligo(dG) as an added template·primer[5], *ts*335 DNA polymerase incorporated about one-third

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Table 1 DNA polymerase activity associated with purified virions of *ts*335 and RSV wild type

Temperature (°C)	Endogenous activity (pmol ^{3}H-dAMP incorporated) *ts*335	RSV(WT)	Ratio of RSV(WT):*ts*335	Exogenous activity with poly(C) oligo(dG) (pmol ^{3}H-dGMP incorporated) *ts*335	RSV(WT)	RSV(WT):*ts*335
34	40	28	0.7	239	315	1.32
37	35	41	1.17	203	375	1.85
40.5	33	61	1.84	68	406	5.97

* The wild type sarcoma virus was the Prague strain (RSV(WT), *ts*335 was isolated as described[1]. Both viruses belong to subgroup C. The virus stocks were grown at 35° C and purified as before[2]. Protein concentrations were determined using the procedure of Lowry *et al.*[23] with bovine serum albumin as a calibration standard. All oligomers used as primers were 12 to 18 nucleotides long.

For the endogenous reaction, assays of heat lability were performed by incubating the purified virions in 0.1 ml of reaction mixture containing 50 mM Tris-HCl, *p*H 8.3, 5 mM dithiothreitol, 6 mM magnesium acetate, 60 mM NaCl, 60 nmol each dTTP, dCTP, and dGTP, 20 nmol ^{3}H-dATP (specific activity 100 c.p.m. pmol^{-1}), Nonidet P-40 to a final concentration of 0.2%, and 12 µg of viral protein. The reaction mixtures were incubated separately at 34° C, 37° C, or 40.5° C for 90 min and radioactivity in acid insoluble material was detected as described before[24]. To assay exogenous activity, purified virions containing 2.4 µg of protein in 0.05 ml of reaction mixture I (100 mM Tris-HCl, *p*H 8.3, 10 mM dithiothreitol, 12 mM Mg acetate, 60 mM NaCl, and 0.2% Nonidet P-40) were incubated at 34° C, 37° C, or 40.5° C for 10 min. After preincubation, to each reaction mixture was added 0.05 ml of reaction mixture II (20 nmol ^{3}H-dGTP, (100 c.p.m. pmol^{-1}), 2.0 µg poly(C) and 0.5 µg oligo(dG)). The reaction mixture was further incubated for 15 min at 34° C, 37° C or 40.5° C and acid-precipitable radioactivity monitored as described before[24].

the amount of ^{3}H-dGMP at 40.5° C as at 34° C, while incorporation by wild type RSV DNA polymerase was increased at 40.5° C (Table 1). Thus, in disrupted virions, the *ts*335 DNA polymerase was more sensitive to thermal inactivation than the wild type RSV DNA polymerase, in agreement with previous data[2].

DNA polymerase was purified from wild type RSV and from the two mutants by standard procedures[6]. Although the enzymes from the mutants were inactivated more extensively than the wild type enzyme during purification and subsequent storage at −70° C in 20% glycerol, the three enzymes behaved indistinguishably during ion-exchange chromatography on DEAE-Sephadex and phosphocellulose.

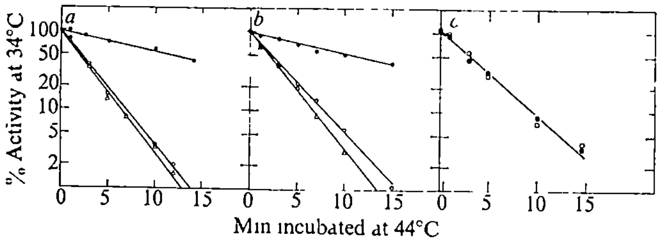

Fig. 1 Thermal inactivation of purified DNA polymerases from mutant and wild type virions using ribohomopolymers as templates. Purified virions of *ts*335, *ts*337 and wild type RSV were obtained as described before[2]. About 4 to 6 mg of viral protein was used for enzyme purification. Virions were lysed and the DNA polymerase purified by chromatography on DEAE-Sephadex and then on phosphocellulose columns as before[6]. DNA polymerase from the mutants *ts*335 and *ts*337 (clone β)[2] and wild type RSV (clone B)[2] eluted at a concentration of 0.06 to 0.08 M KCl from DEAE-Sephadex and 0.24 to 0.30 M KCl from phosphocellulose. A recovery of 40–50% of DNA polymerase was achieved by carrying out the procedures as rapidly as possible. Heat lability was assayed by preincubating the DNA polymerase in reaction mixture I, which contained 100 mM Tris-HCl, *p*H 8.3, 20 mM dithiothreitol, 12 mM magnesium acetate, 120 mM NaCl and 0.05–1.0 U of purified enzyme per 0.05 ml (A unit of enzyme activity is defined as the amount of enzyme required to incorporate 100 pmol of ^{3}H-dGMP at 37° C in 15 min[6].) The reaction mixture was incubated at 44° C. At various intervals aliquots of 0.05 ml were withdrawn and added to 0.05 ml of reaction mixture II which contained template, primer and substrates. This was kept on ice for 10–15 min, then incubated at 34° C for 15 min. Acid-precipitable radioactivity was measured as described[24]. The rate of the enzyme reaction at 34° C was linear for at least 15 min. *a*, Poly (C) (1.0 µg) and oligo (dG) (0.5 µg) were template primer with 20 nmol ^{3}H-dGTP (100 c.p.m. pmol^{-1}) as substrate. ●, RSV wild type; ○, *ts*335; △, *ts*337. *b*, Poly(A) (1.0 µg) and oligo(dT) (0.5 µg) were template primer with 20 nmol ^{3}H-dTTP as substrate. Symbols are as in (*a*). *c*, ●, rabbit reticulocyte 10S RNA (30 nmol nucleotide) and oligo(dT) (140 pmol nucleotide) were template primer with 20 nmol ^{3}H-dGTP (100 c.p.m. pmol^{-1}) and 60 nmol each of dATP, dCTP and dTTP as substrate and DNA polymerase from *ts*337. Reaction mixture II was incubated at 34° C for 60 min. ○, poly(C) oligo(dG) were template primer as in (*a*).

Electrophoresis on SDS-containing polyacrylamide gels followed by staining with Coomassie blue[7], showed that the purified DNA polymerases from *ts*335, *ts*337 and wild type RSV had two polypeptide bands of average molecular weight 88,000 and 56,000. The subunits were in equimolar ratio. In some samples there occasionally appeared a faint band at about 64,000 which we assume to be a contaminant. The purified DNA polymerase from avian myeloblastosis virus (AMV) was not distinguishable from the RSV enzymes. These values for the molecular weights of the polypeptides of the DNA polymerase are somewhat lower than those reported by others[8-10].

The heat-inactivation kinetics of the ability of the purified enzymes to copy ribohomopolymers were assayed using poly(C) oligo(dG) and poly(A) oligo(dT) as template primers. Enzymes were preincubated at 44° C and assayed at 34° C. The enzyme purified from mutant virions was inactivated about three times more rapidly at 44° C than that from RSV wild type virions (Fig. 1*a* and *b*). There was a small reproducible difference between the rates of inactivation of the two mutants, with the *ts*337 enzyme being more heat sensitive.

To test the ability of the enzymes to transcribe RNA heteropolymers, we used 10S RNA from rabbit reticulocytes (globin mRNA) as a template with an oligomer of dT as primer[4]. Figure 1*c* shows that the rate of heat inactivation of the ability of the *ts*337 enzyme to transcribe 10S RNA oligo(dT) was identical to that measured with poly(C) oligo(dG).

When heat-inactivation kinetics were assayed using a deoxyribohomopolymer, poly(dC) primed with oligo(dG), again enzymes from the mutants were inactivated more rapidly than the wild type enzyme. The enzymes from the mutants were inactivated slightly more slowly with this DNA template than with the homologous RNA template of poly(C), while the wild type enzyme was inactivated at the same rate with both templates. The *ts*335 enzyme was again slightly less thermosensitive than the *ts*337 enzyme. Using nuclease-treated calf thymus DNA[11], data indistinguishable from that in Fig. 2 were obtained with enzymes from both mutants and RSV wild type.

We investigated whether reduced binding of the enzyme from the mutants to the template might be partly responsible for their thermolability. Figure 3 shows that if template · primer was added to the reaction mixture during preincubation of DNA polymerases at 44° C, the wild type enzyme was protected against heat inactivation, whereas that of *ts*335 was inactivated at the same rate as in the absence of template. These inactivation conditions, which show such a large difference between wild type and mutant, may be more relevant to the *in vivo* situation than inactivation in the absence of template.

The purified DNA polymerases from *ts*335, *ts*337 and wild type RSV all exhibit, in addition to DNA polymerase activity, a nuclease activity which selectively removes the RNA moiety from a DNA–RNA hybrid (ribonuclease H activity). Figure 4*a* shows the activity of polymerase-associated ribonuclease H from *ts*335, *ts*337 and wild type RSV after incubation at 44° C for various times. The activity from the mutant was more sensitive to temperature than that from the wild type. However, the ribonuclease H activity was less sensitive to inactivation at 44° C than was the DNA polymerase activity[12]. At 47° C the ribonuclease H activity of *ts*337 DNA polymerase was much more thermolabile than the wild type enzyme (Fig. 4*b*).

The half lives of the synthetic and degradative activities of purified DNA polymerase from the mutants and the wild type RSV are collected in Table 2. The enzymes from the mutants were four to ten times more heat labile than those from the wild type virus.

Revertants and recombinants

Mason *et al.* (in preparation) isolated revertants of both *ts*335 and *ts*337 which are no longer temperature sensitive for the initiation of infection at 41° C. To see if the phenotypic reversion was paralleled by a reversion of DNA polymerase to wild type behaviour, the heat inactivation kinetics of the DNA polymerase activity in virions of revertants of *ts*335 and *ts*337 were compared with that of mutant *ts*337 utilising poly(C) oligo(dG) and poly(dC) oligo(dG) as template primers. The virion-associated DNA polymerase activity of the revertants was three or four times more heat stable than that of the mutants (Fig. 5*a* and *b*). Similarly, the ribonuclease H activity of the DNA polymerase of the revertants was ten times more resistant to thermal inactivation than ribonuclease H activity from the mutants (Fig. 5*c*). Therefore both the biological and biochemical parameters vary together during selection for reversion of the temperature sensitive lesion.

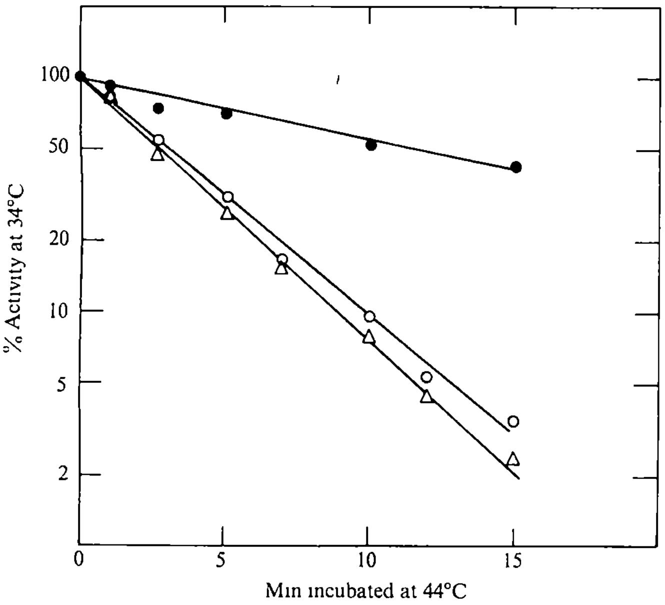

Fig. 2 Thermal inactivation of the DNA polymerase activity of mutant and wild type enzymes with poly(dC) oligo(dG) as template. The same reaction conditions were used as described in the legend to Fig. 1*a*, except that 1.0 μg of poly(dC) was substituted for poly(C). ●, RSV wild type, ○, *ts*335, △, *ts*337.

Because the mutants were isolated from a transforming virus of host range subgroup C Prague RSV, recombinants could be selected from mixed infections with the group B non-transforming leukosis virus, RAV-6[13,14]. Recombinants were selected as transforming viruses of group B. The temperature-sensitive lesion was an unselected marker because permissive temperature was used throughout the selection. Recombinant clones were then screened for temperature sensitivity of growth and transformation (W. S. M., R. R. Friis, M. Linial and P. K. Vogt, in preparation).

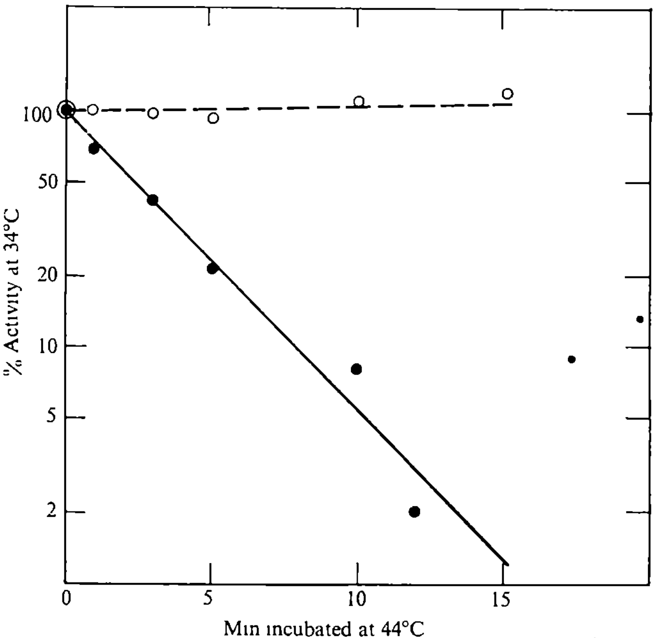

Fig. 3 Effect of template primer on the rate of heat inactivation of mutant and wild type RSV DNA polymerase activity. The reaction was as described for Fig. 1*a*, except that template primer was included in reaction mixture I. ○, RSV wild type, ●, *ts*335.

Heat inactivation kinetics of the DNA polymerase activity in the virions of the wild type and temperature sensitive recombinants were assayed (Fig. 6). Both synthetic and degradative activities of the DNA polymerases of the wild type viruses were more thermostabile than those of enzymes of the temperature sensitive recombinants.

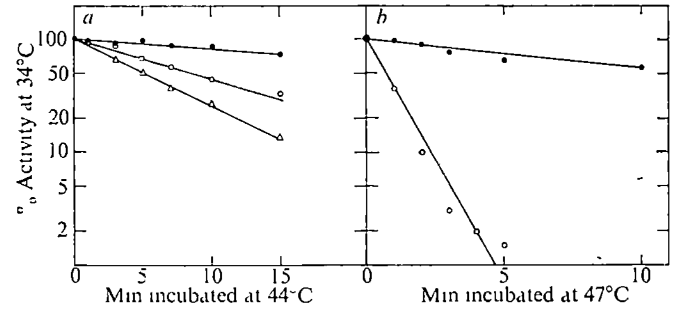

Fig. 4 Thermal inactivation of ribonuclease H activity of mutant and wild type RSV DNA polymerase. Ribonuclease H was assayed by using ^{3}H-poly(A) poly(dT) as substrate and monitoring the degradation of ^{3}H-poly(A) to acid-soluble material. Heat inactivation was carried out as for Fig. 1 by preincubation at either 44° C or 47° C followed by incubation at 34° C. Reaction mixture I was as for Fig. 1*a*, except that 40 mM Mg acetate and 60 mM NaCl were used. Reaction mixture II contained 10 pmol of ^{3}H-poly(A) (1,000 c.p.m. pmol^{-1}) and 2.8 pmol of poly(dT). The reaction at 34° C was carried out for 60 min under an atmosphere of nitrogen. The acid-soluble radioactivity was determined by first adding 10 μl of 10 mg ml^{-1} bovine serum albumin and 30 μl of 50% trichloroacetic acid to the reaction mixture at the termination of incubation. After 15 min at 4° C, the samples were centrifuged in a bench-top centrifuge at full speed for 3 min and 100 μl aliquots were withdrawn from the supernatant and counted directly in 5.0 ml of dioxane-based scintillation fluid. The degradation of poly(A) by the unincubated enzyme is represented as 100%. All other values are normalised to the value of the unincubated sample. *a*, ●, RSV wild type, ○, *ts*335, △, *ts*337. *b*, ●, RSV wild type, ○, *ts*337.

Table 2 Average times for inactivation of half of the activities of the purified DNA polymerase from mutant and wild type RSV

Source	DNA polymerase activity at 44° poly(C) oligo(dG)	 poly(dC) oligo(dG)	Ribonuclease H activity at 44° C	 47° C
RSV wild type	11 5	11 5	30	13
*ts*335	2	3	7 4	—
*ts*337	1 8	2 8	5	1 5

Values are expressed in min as $t_{1/2}$, which is the time required for inactivation of 50% the relevant activity as determined in Figs 1, 2 and 4

Implications of thermolability

RNA tumour viruses contain a DNA polymerase which can utilise polyribonucleotides as templates to synthesise a faithful complementary DNA transcript[4] This enzyme has been attributed a central role in the provirus hypothesis[15] In the scheme of events postulated in the provirus hypothesis, such an enzyme needs to be operational just after infection for the synthesis of DNA complementary to viral RNA However, it has not previously been possible to determine unambiguously if the virion DNA polymerase is responsible for provirus synthesis and if the enzyme is coded by the viral genome or is cellular in origin Virus-specificity of the enzyme has been suggested because it appears only after infection of the cell[16,17] The finding of noninfectious virions lacking DNA polymerase has been interpreted as evidence for an obligatory role for the enzyme[18-21]

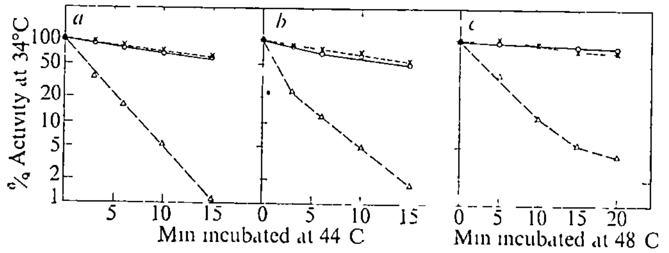

Fig 5 Thermal inactivation of polymerase and nuclease activity associated with purified virions of revertants of *ts*335 and *ts*337 Spontaneous revertants of *ts*335 and *ts*337 were selected by growth of the temperature sensitive viruses at the non-permissive temperature (41° C) Cloned revertant stocks were then isolated at the permissive temperature (35° C) For the experiments reported in this figure, 7 0 μg of viron protein from the following stocks was used *ts*335 revertant clone A-3, *ts*337 revertant clone γ-2 and *ts*337 clone γ (W S M , R R Friis, M Linial and P K Vogt, in preparation) *a*, Inactivation of polymerase activity measured with poly(C) oligo(dG) as in Table 1 *b*, Inactivation of polymerase activity measured with poly (dC) oligo(dG) as in Table 1 *c*, Inactivation of ribonuclease H activity measured as in Fig 4 Preincubation was at 48° C ×, *ts*335 revertant, ○, *ts*337 revertant, △, *ts*337

The study reported here demonstrates covariation of thermolability of the virion DNA polymerase with temperature sensitivity of infection and transformation by the virions Two early temperature sensitive mutants of RSV were found to have DNA polymerase more thermolabile than that of the wild type virus, while revertants of the mutants (W S M , R R Friis, M Linial and P K Vogt, in preparation) as well as a class of wild type recombinants[2] had DNA polymerase with wild type properties This indicates that RSV RNA encodes the information for at least part of the DNA polymerase molecule and the enzyme must be necessary to initiate infection or transformation by RSV Bishop *et al* [27] reported that less viral DNA is produced by *ts*335 and *ts*337 at nonpermissive temperature than is produced by the wild type virus, which implies that the virus-encoded DNA polymerase is responsible for the synthesis of DNA after infection by RNA tumour viruses

All three activities of the purified DNA polymerase from avian tumour viruses show increased thermolability in the DNA polymerase of the mutant viruses, and therefore seem to reside in the same polypeptide chain Grandgenett *et al* [8] have shown that the isolated, lower molecular weight subunit of AMV DNA polymerase manifests both the synthetic and the degradative activities of the whole enzyme We have confirmed this for RSV and have isolated the small subunit of the DNA polymerase from *ts*337 and wild type virus Both synthetic and degradative activities of the isolated small subunit of *ts*337 showed heat-inactivation kinetics indistinguishable from those of the complete *ts*337 DNA polymerase (I M V , A Panet and W S M , in preparation) The lower molecular weight subunit must therefore be encoded by viral RNA, the origin of the other subunit is under investigation

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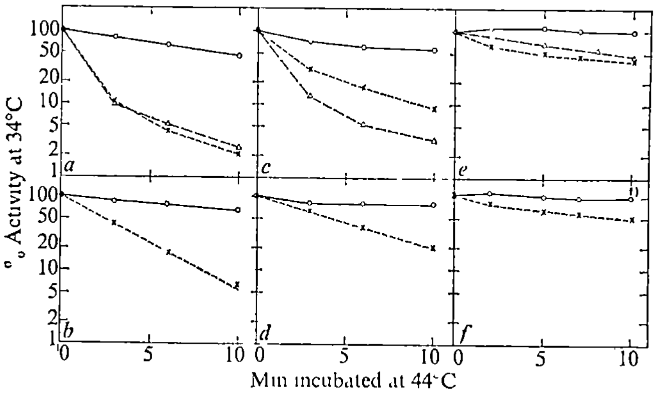

Fig 6 Thermal inactivation of DNA polymerase and ribonuclease H activity of various stocks of recombinants Recombinants between temperature sensitive RSV stocks of subgroup C and avian leukosis virus RAV-6 of subgroup B, were isolated as described (W S M , R R Friis, M Linial and P K Vogt, in preparation)[3] The recombinant sarcoma viruses had the subgroup B host range characteristic of RAV-6 and were of either wild type or temperature sensitive genotype We have used the following nomenclature *ts*337 × RAV(WT) or *ts*335 × RAV(WT) the mutant virus was co-cultivated with RAV-6 and the resultant viruses after appropriate screening could transform and replicate at non-permissive temperature (41° C) The following clones were used to prepare virus stocks *ts*337 × RAV(WT), clone 21, *ts*335 × RAV(WT), clone 17 (in preparation as above) *ts*337 × RAV(Ts) or *ts*335 × RAV(Ts) the mutant virus was cocultivated with RAV-6 and the resultant viruses after appropriate screening could not transform or replicate at nonpermissive temperature (41° C) The following clones were used to make virus stocks *ts*337 × RAV(Ts), clone 7, *ts*335 × RAV(Ts), clone 14 (in preparation as above) *a*, Poly(C) oligo(dG) was template primer ○ *ts*337 × RAV(WT), ×, *ts*337 × RAV(Ts), △, *ts*337 *b*, Poly(C) oligo (dG) was template primer 0, *ts*335 × RAV (WT), X, *ts*335 × RAV (Ts) *c* and *d*, Poly (dC) oligo (dG) was template primer Symbols as in *a* and *b*, respectively *e* and *f*, Ribonuclease H assays Symbols as in *a* and *b*, respectively

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Membrane structure changes in rod outer segments associated with rhodopsin bleaching

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Static and time resolved birefringence analysis is used to quantitate a fast membrane structure change driven by the formation of metarhodopsin II in intact frog retina. This change can be accounted for by the disorientation of only one phospholipid molecule per rhodopsin bleached.

SCHMIDT'S discovery and analysis of the radially positive birefringence of myelin sheath and of the positive uniaxial birefringence of retinal rod outer segments (ROS) gave early evidence of a two-dimensional liquid crystalline structure for biological membranes[1]. More recently, time-resolved birefringence measurements have been made in attempts to detect structural alterations, such as lipid reorientation, suspected to accompany action potentials in excitable membranes. Very small transient changes from the resting birefringence associated with action potentials of nerve, electroplax and muscle have been detected, usually by summing many responses in a signal averager[2–6]. Where sufficiently extensive analysis of these responses has been possible, however, the birefringence transients have been found to be secondary to the excitation process rather than part of its mechanism[7]. This does not mean that there is no primary alteration of membrane anisotropy, but rather that the surface density of sites at which such changes occur in these tissues may be too small to be detected, or may be small compared with the background of less interesting changes.

It is not surprising that little effort has been made to look for small changes in the birefringence of rods coincident with excitation, since the light used to observe the birefringence may at the same time alter the system by bleaching its pigment. Nevertheless, our experience using infrared image converters and sensitive photomultipliers to deal with dark adapted ROS[8], suggested that a birefringence analysis of this material might be feasible and would be especially worthwhile for the following reasons.

Individual frog ROS contain 2.5 mM rhodopsin in an 1,800 μm^3 volume[9]. Thus 3×10^9 equipotent trigger molecules[10] are embedded in the 4,000 closely stacked lamellar membranes (2,000 disks), each of 28 μm^2 area to yield a surface density of 25,000 rhodopsins per μm^2. This, for example, exceeds by 1,000 times the density of sodium channels in nerve[11]. The 60-μm long ROS themselves lie parallel to one another in a dense array without intervening inactive material (cytoplasm, mitochondria, Schwann sheath, myelin) so predominant in nerve. In sum, the features of very high active membrane density and good alignment of extensive material of high purity provide a most appropriate subject for birefringence analysis.

ROS birefringence transient

Using an infrared dissecting microscope, dark adapted retinae from *Rana pipiens* were isolated in frog Ringer solution. For static birefringence studies, outer segments were dislodged on a microscope slide by gently tapping a piece of retina against the slide. A drop of Ringer or 12% gelatin-Ringer solution was added, the preparation closed with a cover-slip and the edges blotted dry and sealed with paraffin wax as before[12]. For kinetic studies, the retina was cut into a dozen or more strips, each of which curled spontaneously to form a tube with the ROS outermost. Each was transferred to a microscope slide and surrounded by a paper spacer to prevent squashing when the cover-slip and wax were added. These strips form so-called 'edge-fold'

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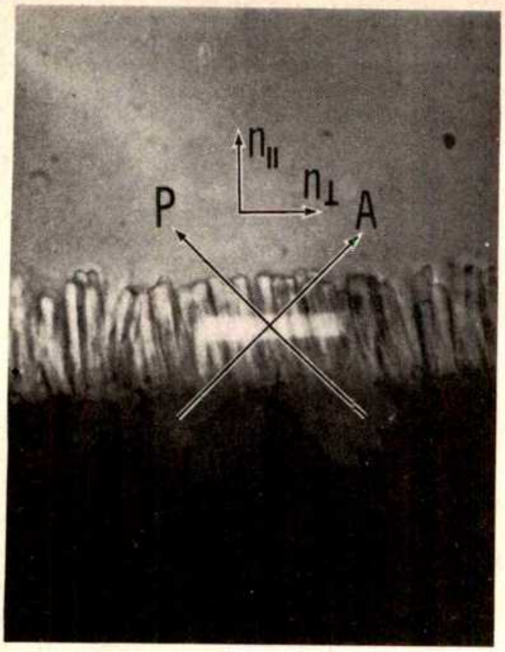

Fig. 1 Edge-folded retina showing aligned rows of rod outer segments with measuring beam superimposed. For birefringence measurement, light initially polarised at a 45° angle (P) with respect to the ROS axis traverses the cell. Since $n_{\parallel} > n_{\perp}$, the phase of the electric field component parallel to the rod axis is retarded with respect to the component perpendicular to it. An analyser (A) transmits only the minor axis component of the resultant elliptically polarised light emerging from the ROS. The birefringence, $\Delta n = n - {}_{\parallel}n_{\perp}$, is defined as axially positive when $n_{\parallel} > n_{\perp}$.

preparations in which aligned rows of ROS project from the edge of the retina[13]. Birefringence observations were made using a low numerical aperture polarising microscope equipped for infrared viewing and with an inverted objective lens as a condenser. A low intensity measuring beam of monochromatic light (30 μm × 300 μm at the specimen plane) was imaged on the ROS layer (Fig. 1). A 1-ms xenon bleaching flash beam was demagnified to the same dimensions as the measuring beam and superimposed on it using a beam splitter. The flash was made blue-green by a Wratten 47B filter together with a series of infrared absorbing glass filters. Its intensity was sufficient to bleach 50–60% of the pigment with each flash, a saturating bleach[14,15]. The flash was blocked from the photomultiplier by a red-transmitting cutoff filter. Birefringence transients initiated by the flash were detected as a change in measuring beam intensity through a system of crossed polariser and analyser with the specimen in between. The axis of the ROS was at an angle of 45° with respect to the polariser axis (Fig. 1). Transmitted intensity was recorded using a storage oscilloscope directly coupled to the amplified output of the detecting photomultiplier (S-20 cathode).

To avoid problems of interpretation due to visual pigment absorption changes on bleaching, flash-induced birefringence changes were initially sought at wavelengths longer than 650 nm, outside the absorption band of rhodopsin. Exposure to a saturating flash initiated a rapid 6–8% loss of light transmission, corresponding to a 3–4% decrease in the net birefringence (Fig. 2). Flashes of reduced intensity caused proportionally smaller changes. The transmission loss had the action spectrum of rhodopsin. A 1/20λ retardation plate inserted between polariser and specimen served both to calibrate the instrument and to prove that the signal measured was a change in birefringence. When this plate was positioned so as to add to the specimen birefringence, the flash-induced signal was a reduction of transmitted light intensity. When the plate was rotated to produce a total static retardation of the same magnitude but of opposite sign, the signal was inverted. This shows that the flash-induced signal was not due to absorption, optical rotation or light scattering changes in the specimen, but that it was due to a change in birefringence.

Wavelength dependence

In experiments using measuring lights of different wavelength, the birefringence transient (BRT) was found to be achromatic at wavelengths between 700 nm and 650 nm, to vanish between 650 nm and 600 nm and to be reversed in sign (birefringence increase) from 600 nm to 550 nm, increasing in magnitude with decreasing wavelength. This peculiar wavelength-dependent behaviour of the BRT was understood through measurement of the static wavelength dependence of the birefringence of single dark adapted ROS. Our dual beam low-light-level wavelength scanning microspectrophotometer[8] was altered by insertion of crossed polarisers and a 1/20λ retardation plate to allow birefringence recording. The logarithm of the transmission ratio between a region containing the retardation plate alone and that containing a single ROS plus retardation plate was measured using microbeams of cellular dimensions without detectable visual pigment bleaching. Figure 3*a* shows the results of such a recording with the slow axis of the retardation plate parallel to the ROS long axis (the birefringence slow axis). Reversal of the retardation plate orientation leads to mirror inversion of the recorded spectrum, indicating that the entire spectrum is due to birefringence. The effect of fractional bleaches of the visual pigment is immediately to reduce the peak-to-peak amplitude of the spectral excursions. A slow achromatic increase in birefringence also occurs subsequent to bleaching with a half-time of about 20 min at 20° C. The retardation spectrum in Fig. 3*b* was computed from the recorded transmission spectrum using the formulae derived by Szivessy[16]. The resulting curve can be understood in the following way. When matter interacts

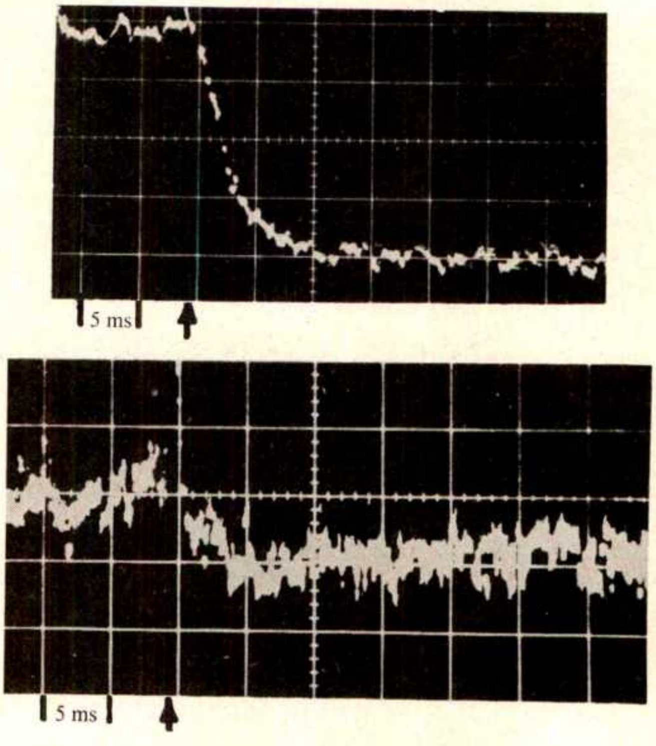

Fig. 2 Comparison of metarhodopsin II (MII) formation and the BRT. The upper oscilloscope trace shows the *in situ* loss of light transmission at λ=380 nm due to a flash induced (at arrow) increase in MII concentration. The temperature was 21.5° C. The lower trace shows the BRT at λ=680 nm under the same conditions. The temperature was 21.8° C.

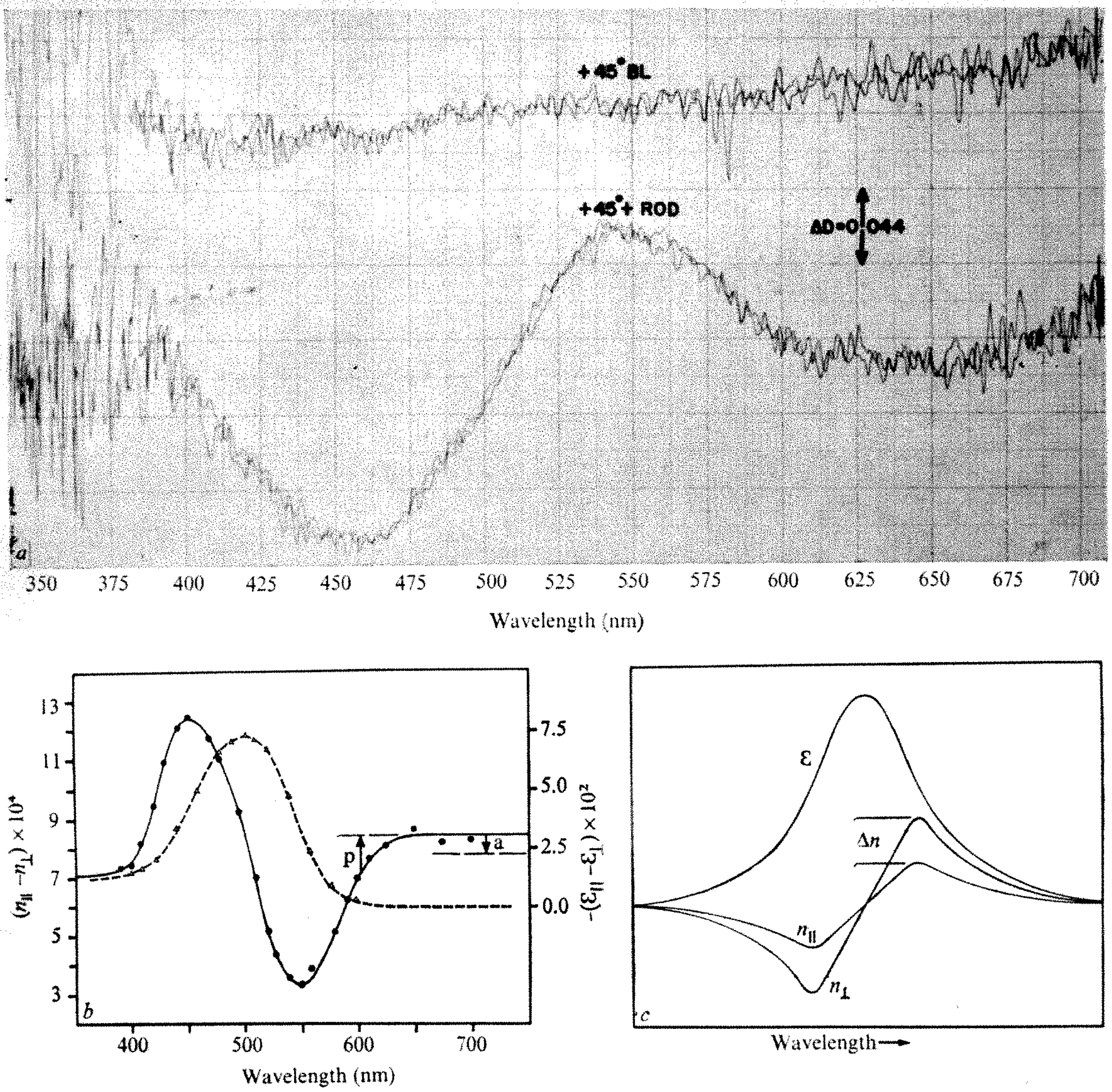

Fig. 3 *a*, Wavelength dependence of transmitted light intensity due to dark adapted ROS static birefringence plus a λ/20 plate aligned with its slow axis along the ROS axis, 45° from the polariser axis (+45° + ROD). Superposition of two successive scans shows that no bleaching occurs during the measurement. Baseline taken in the absence of the ROS (+45° BL). Intensity increases downward. ΔD is the equivalent optical density change. *b*, Birefringence spectrum (●) computed from data of (*a*). Arrow a indicates the flash-induced achromatic birefringence loss. Arrow p indicates the wavelength-dependent birefringence change due to loss of visual pigment related anomalous dispersion differences. The direction and magnitude of the BRT depends on the relative magnitudes of a and p at a given wavelength. Dichroism spectrum (△) showing a higher absorption coefficient for the perpendicular (to the rod axis) component than for the parallel component, the maximum difference occurring at the absorption peak of rhodopsin. *c*, Schematic diagram showing that the anomalous dispersion of the refractive index in the region of the rhodopsin absorption peak is more pronounced for the perpendicular direction, $n_{\perp}$, than for the parallel direction, $n_{\parallel}$, due to dichroism of the visual pigment. For wavelengths longer than the absorption peak, the anomalous dispersion-dependent birefringence component is negative ($n_{\parallel}-n_{\perp}<0$). Loss of this negative component on bleaching contributes an increase to the net birefringence.

with light, in the region of an absorption band, there can be both absorption of photons and a phase change (retardation) of the transmitted wave. These properties are expressed as the imaginary and the real part of the complex index of refraction respectively. The two components of the complex index are mathematically related by the Kramers-Kronig or other dispersion relationships[17,18]. In spectral regions distant from the absorption peak, the real part of the refractive index, *n*, decreases with increasing wavelenght ($dn/d\lambda<0$). However, in the region of the absorption peak, *n* increases with increasing wavelength ($dn/d\lambda>0$), producing an anomalous dispersion effect. Intact ROS are dichroic because of the net orientation of rhodopsin chromophores in the membrane. Absorption of light polarised perpendicular to the rod axis (in the plane of the lamellar membranes) is four to five times more efficient than of light polarised parallel to the rod axis[12]. Similarly, this means that the anomalous dispersion associated with rhodopsin absorption will be more pronounced for the perpendicular component of the refractive index, $n_{\perp}$, than for the parallel component, $n_{\parallel}$, as shown schematically in Fig. 3*c*. Our birefringence spectrum in the region of the absorption peak measures the difference, $\Delta n=n_{\parallel}-n_{\perp}$, between the dispersion curves for the two polarisations. Note that this anomalous dispersion component of the overall birefringence is negative with respect to the rod axis for wavelengths longer than the absorption peak.

Possible sources of BRT

Could the disappearance of the visual pigment anomalous dispersion effect as a result of bleaching then explain the observed long-wavelength BRT. Evidently not, for this loss of negative birefringence would cause an increase in the (positive) net birefringence whereas a decrease is actually observed. If the long-wavelength BRT, however, is due to the concurrent loss of a wavelength-independent positive contribution to the net birefringence, such as would

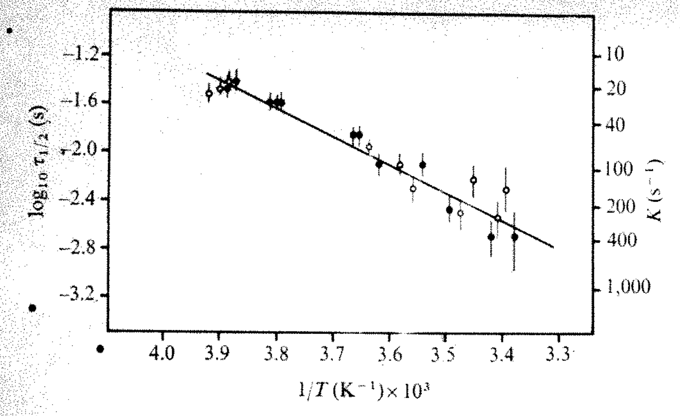

Fig. 4 Arrhenius plots of the half-times of MII formation (○) and of the BRT (●) for an edge-folded retina. The equivalent first order rate constant scale is on the right.

be associated with a lipid phase transition of the disk membranes, or to the gain of a negative one, we would expect to find some shorter wavelength at which the gain in birefringence due to loss of the anomalous dispersion effect would be just offset by the loss in birefringence caused by the achromatic component. This is in fact the case, as described above.

Although this analysis shows that the achromatic BRT cannot be attributed to a loss of the visual pigment anomalous dispersion effect, its millisecond relaxation time suggested that it is linked to the formation of the photoproduct metarhodopsin II (MII), which appears at a similar rate in solution[91]. To test this hypothesis we measured the half-times of *in situ* MII formation and of the BRT (Fig. 2) as a function of temperature, using edge-folded retina preparations mounted on a theromostatically controlled Peltier cooler on the microscope stage. Coincidence of the Arrhenius plots of these measurements is taken to support the notion that the BRT is closely linked to the formation of MII (Fig. 4). Formation of MII might only be a rate-limiting trigger, however, and the nature of its linkage to the BRT remained to be explored.

In studies on bleached ROS, Schmidt showed that the total static birefringence is the sum of two opposing components, the form and the intrinsic birefringence, $n_T = n_F + n_I$. A body constructed of layers of membrane alternating with cytoplasm must have a negative form birefringence, independent of considerations of molecular orientation, that is quantitatively predicted by Wiener's equation[20]:

$$(n_{\parallel} - n_{\perp})_F \equiv \Delta n\mathrm{F} = -[f_c f_m (n_m{}^2 - n_c{}^2)^2]/[2n_T(f_c n_m{}^2 + f_m n_c{}^2)] \quad (1)$$

where Δn_F is the form birefringence, f is volume fraction, n is refractive index and the subscripts m, c, and T refer to membrane, cytoplasm and mean index for the whole body, respectively. Schmidt reasoned that since ROS show net positive birefringence ($n_{\parallel} > n_{\perp}$), there must also be an intrinsic component that is positive and of greater magnitude than the negative form component. He concluded that the intrinsic component resulted from net orientation of the lipid hydrocarbon chains perpendicular to the plane of the membrane, giving a higher refractive index along the chains than perpendicular to them. This constituted the discovery of membrane 'crystallinity'.

From the Wiener equation it is easy to see that a small change in the cytoplasmic volume fraction, f_c, could cause Δn_F to change. This might be mediated by an MII-driven change in osmotic pressure of the cytoplasm secondary to ion flow or a chemical dissociation reaction. It has also been shown, using X-ray diffraction techniques, that at some time during the 4–8 h after bleaching, rhodopsin has sunk from a position of $\frac{1}{3}$ immersion to a position of $\frac{1}{2}$ immersion in the frog lamellar membranes[21]. Since the membrane refractive index, n_m, is due to both lipid and embedded protein, and the protein refractive index is higher than that of lipid, increased protein immersion on bleaching would cause n_m to increase at the expense of n_c. Wiener's equation predicts that such a redistribution of refractive index components would cause an increase in the magnitude of the negative form birefringence, again consistent with our measured result if this change were driven by the formation of MII. Alternatively, a change in disk membrane surface potential concurrent with MII formation such as could be associated with an ionic redistribution or the early receptor potential, might cause compression and/or expansion of the membrane or a Kerr effect dipole realignment, as has been postulated for nerve by Cohen *et al.*[2,6,7]. This could change n_m, f_m or both.

To determine whether ionic mechanisms might be involved in either osmotic or surface potential effects, we looked for the BRT in retinae immersed in Ca^{2+}-free, Na^+-free, K^+-free and Cl^--free media and in media containing tetrodotoxin or ouabain. The BRT during all these treatments was indistinguishable from normal. In addition, the BRT persisted in frozen (< −10° C) and in glutaraldehyde-fixed retinae. Clearly, the BRT is not dependent on shifts in water distribution or on ionic diffusion potentials, known to be important in nerve. We therefore ruled these out as causal agents.

To examine the possibility that the BRT is caused by rhodopsin sinking into the lamellar membrane concurrent with MII formation, we note the finding of Korenbrot *et al.*[22] that glycerol readily enters ROS and permeates the cytoplasmic space. Using the glycerol imbibition method, we did index matching experiments, analogous to those done by Blasie using X-rays, with the advantage of the millisecond time resolution of the birefringence measurement. Retinae or single ROS were equilibrated in a series of Ringer solutions containing increasing fractions of glycerol. Since the refractive index of glycerol is 1.47, the

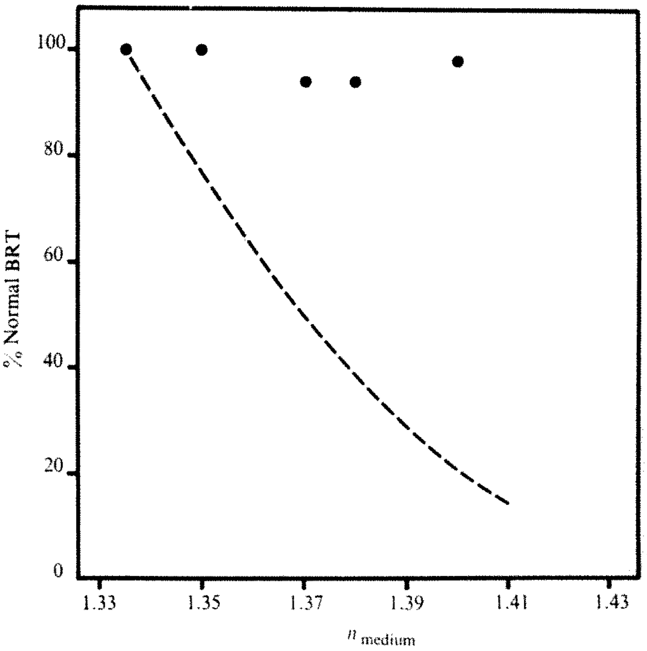

Fig. 5 Effect of glycerol imbibition on the relative magnitude of the BRT. Static form birefringence measurements indicate that, if the BRT was due to a change in form birefringence, the experimental points should lie on the dashed line. Lack of dependence of the BRT on imbibing medium refractive index, n_{medium}, shows that the achromatic BRT is due to a loss of intrinsic birefringence.

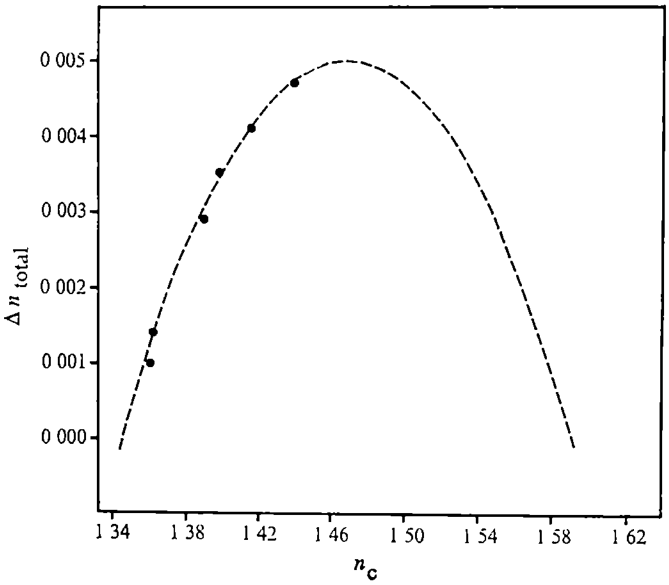

Fig 6 Static birefringence imbibtion curve of single ROS Experimental points (●) lie on a plot of the Wiener equation for which the following best fit values have been used n_m=1 475, n_c=1 365, f_m=0 37 and f_c=0 63 The curve shows that the net static birefringence of +0 0010 in Ringer solution is the sum of +0 0050 intrinsic component and a −0 0040 form component

effect of these treatments was progressively to diminish refractive index contrast between membrane and cytoplasm as the cytoplasm index increased If the BRT is caused by refractive index redistribution due to sinking of part of the rhodopsin molecule from cytoplasm to membrane, the influence of this redistribution should be diminished as the cytoplasmic index increases, up to the point were the index of the cytoplasm (glycerol) matches that of the non-rhodopsin part of the membrane At match, a sinking rhodopsin can only move between two media of identical refractive index and its effect on the birefringence should then be eliminated The results of these experiments show that the BRT is independent of the cytoplasmic refractive index and thus of the magnitude of the form birefringence component (Fig 5) This rules out the sinking of rhodopsin as a cause of the BRT More importantly it shows that the bleach-induced sinking of visual protein inferred by Blasie cannot occur on a time scale concurrent with visual excitation The hypothesis that visual protein might float on one surface of the membrane in darkness and then, on light excitation, sink to bridge the lipid phase with a pore, allowing Ca^{2+} to escape, is therefore not supported by our measurements

The experiments described here provide strong evidence that the achromatic BRT signals either lipid reorientation or a change in protein conformation, or both A small negative activation entropy calculated from our Arrhenius plots of MII formation argues against a sizeable membrane conformation change, although it is difficult to say how much would be enough It is also conceivable that compensating entropic changes occur in different parts of the lipid-protein complex, thus masking the true size of a conformational transition One of these changes might be optically anisotropic while the other is not

Birefringence and rod structure

We were able to learn something further of rod structure by measuring their static birefringence in glycerol-containing media of increasing refractive index The result (Fig 6) constitutes a so-called imbibition curve whose form is given by the Wiener equation As described above, glycerol imbibition allows the form birefringence to be eliminated progressively It not only unmasks the positive intrinsic birefringence previously compensated by the nearly identical negative form birefringence, but it allows the values of n_c, n_m, f_c and f_m to be determined From these measurements we find Δn_F to be −0 0040 for dark adapted *Rana pipiens* rods Since we measure the total birefringence of normal dark adapted cells to be +0 0010, the intrinsic birefringence, Δn_I, is +0 0050 Other values obtained by fitting the curve to Wiener's equation are n_m =1 475, n_c =1 365, f_m =0 37 and f_c =0 63 Since the refractive index for Ringer solution is 1 336, the value n_c =1 365 suggests that the cytoplasmic space contains about 16% solids, based on the mean refractive increment for sugars and proteins of ~0 016 per g % (ref 23) More than half of this value can be accounted for by protrusion of rhodopsin molecules from the membrane surface into the cytoplasmic space, while the remainder might be due to the protruding saccharide groups of glycolipids Cytoplasmic bridges between disk membranes have been reported[24], and similar structures have been postulated to account for the restorative force in osmotic shrinkage experiments on ROS[25] The cytoplasmic volume fraction, f_c, is in reasonable agreement with estimates of other workers[22,26]

It is useful to compare our value for the intrinsic birefringence of ROS, +0 0050, with that of myelin, +0 011 (ref 27) Although the two-fold difference suggests a more crystalline lipid order in myelin, it must be remembered that the large cytoplasmic volume fraction of ROS dilutes the measured membrane birefringence by more than a factor of two[28] Thus the true membrane value for ROS is about the same as for myelin In view of the higher degree of unsaturation of ROS membrane phospholipids[29,30] and low cholesterol content[31], this result is rather surprising and implies that ROS protein may impose a more crystalline order on the ROS lipids than would occur in an equivalent protein-free bilayer Alternatively, rhodopsin may itself be birefringent

Implications

If the BRT is due to a loss in crystallinity of the lipids of ROS membranes, it is interesting to learn what fraction of the total crystallinity it represents The ratio of the BRT to the intrinsic birefringence is about 1% when extrapolated to complete rhodopsin bleaching Since there are about 100 phospholipid molecules per rhodopsin in ROS disk membranes[32], the BRT could, therefore, be due to the disorientation of only one phospholipid molecule per rhodopsin bleached Alternatively, a small anisotropic change in protein structure as MII is formed might account for our results

On the basis of changes in ultraviolet absorption[33,34], ultraviolet circular dichroism[35,36], chemical reactivity[37,38] and molecular size[39] of detergent-solubilised rhodopsin, previous workers have concluded that a change in protein conformation accompanies bleaching Nevertheless, circular dichroism measurements on rod suspensions fail to confirm this evidence when rhodopsin is in its native milieu[40,41] Though flash photolysis measurements have been said to point independently to a conformation change during MII formation because of a large positive activation entropy found with this step[42,45] such measurements have been confined largely to detergent-solubilised rhodopsin or to suspensions of previously frozen or otherwise aged rod particles Contemporary evidence suggests that both these conditions alter the activation parameters found in fresh material[46] and we in fact find a negative activation entropy in our work on fresh rods Thus, it is difficult to judge the significance of either the very specific assays of conformation change such as circular dichroism or of the less specific activation energy analyses for our work In seeking an explanation for the BRT in intact retinae, however, it seems that a change in protein conformation must occur, for without such a prime mover, there would be no evident trigger for a lipid reorientation. On the other hand, if the BRT does not signal lipid reorientation, it must be directly detecting a protein conformation change.

There is keen interest in the use of biophysical techniques to detect and identify dynamic properties associated with physiological function of cell membranes or their isolated constituents. The ideal detection technique would be sensitive, time resolved, and specific for a particular molecule or molecular property. Measurements would be possible on intact cells without significant perturbations in structure through introduction of extrinsic probes. Needless to say, the ideal does not exist in a single technique and choice is frequently dictated by problems peculiar to each tissue.

Though it is easy to fault birefringence analysis on the basis of poor specificity, we have found in its application to rod outer segments a technique that is sensitive, time resolved, without perturbation and applicable to intact cells. In addition, specificity may be designed into the experiment. It has thus been possible for us to detect a membrane structural alteration in intact visual receptors that is specifically linked to one stage in pigment bleaching and that is fast enough to be associated with visual excitation.

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letters to nature

Large outbursts in Cygnus X-3

Two radio outbursts in Cyg X-3 were observed during December 1973 and January 1974. The source was observed at 4.2 GHz using the 26-m radio telescope of Kashima Branch of the Radio Research Laboratories, which has a half power beamwidth of 10 arc min. Flux densities were obtained using drift scan so as to avoid interference from HII regions surrounding Cyg X-3. An average of four or five scans was taken to determine the flux densities. The receiver gain was calibrated during each determination using the output from a noise tube. It was also calibrated at five hour intervals by observing 3C147 (assumed flux density 9.3 Jy—1 Jansky=1 f.u.—) and 3C274 (80 Jy) in December 1973, and 3C123 (19.0 Jy) in January 1974. We measured the total flux densities alternately in orthogonal planes of polarisation by rotating the polariser, except on December 23 and 24, 1973. On those occasions the flux densities were measured in only one plane of polarisation. The pointing accuracy was checked frequently by the scans with declination offset. The error in flux density is less than $0.3+0.05S_{4.2}$ Jy, where $S_{4.2}$ is the flux density at 4.2 GHz.

An outburst was first recognised at 2200 UT on December 23, 1973, with a flux density of about 6.5 Jy. On December

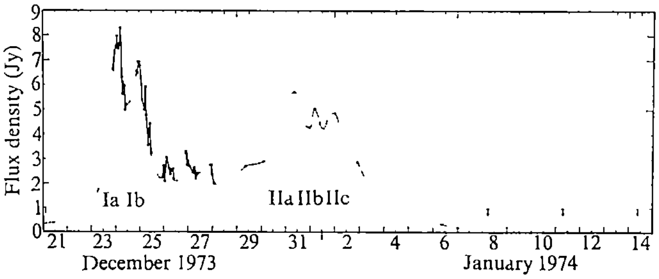

Fig 1 Observed variation in the flux density of Cyg X-3 at 4 2 GHz Peaks are numbered Ia–IIc

21 the flux density of the source had been less than the noise level (upper limit 1 Jy) Five hours after it was first detected, the flux density reached its maximum of 8 30 Jy (Fig 1) Seven days after the first event, a second outburst was observed The second outburst was observed only a few times a day

The two outbursts occurred about a week apart and both had a few peaks, each lasting about a day (Fig 1) Both of these features are similar to those observed during the outbursts of Cyg X-3 in September 1972 (refs 1–3)—before now the only large event known to have occurred on the source since its radio identification on July 19, 1971 (ref 4) It has, however, been monitored at intervals of a few months at Kashima since January 31, 1973, and except for this event, the flux densities have not exceeded 1 Jy

We thank F Takahashi and T Miki for their collaboration This work was one of the cooperative observations between Radio Research Laboratories, Japan, and the Tokyo Astronomical Observatory, University of Tokyo

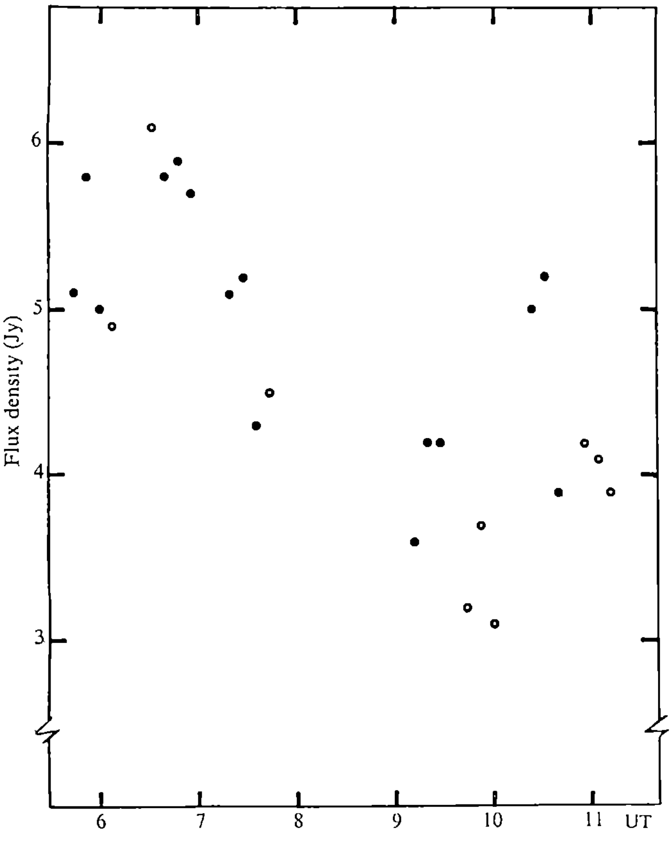

Fig 2 Fine structure of the intensity variation on December 25, 1973, showing the spikes on peak Ib at 0644 and 1100 UT, respectively Each point was obtained from one drift scan Filled circles, observations at 0° (vertical) of the telescope polariser angle, open circles, 90° (horizontal) of the telescope polariser

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Timing results for thirteen pulsars

As part of a continuing programme of pulsar timing measurements at the Arecibo Observatory, timing results have been obtained for thirteen pulsars following a year or more of observations at 430 MHz Precision timing results for these thirteen objects have not been reported previously The discovery names, references, and relevant least-squares fitting information are given in Table 1 The fitted parameters right ascension, declination, period (P) and period derivate ($\mathrm{d}P/\mathrm{d}t$) are listed in Table 2

The techniques used in the observations were basically the same as in previous work at Arecibo[1,2] Arrival times were determined by the template-matching method[2], the template in the present case being the sum of a large number of pulses processed by an incoherent de-dispersing device[3] The error bars assigned to daily average arrival times are the standard deviation from the mean of all the arrival times (usually ten or more) within the day Ephemeris corrections were carried out using data supplied by the Massachusetts Institute of Technology Lincoln Laboratory and were based on the work of Ash, Shapiro and Smith[4] The errors assigned to the fitted parameters in Table 2 are twice the formal standard errors resulting from the least-squares fitting process, the latter being computed according to Jurgens (unpublished) in order to reflect explicitly the value of χ^2

There are several aspects of the results which require comment Fits for several of the pulsars yielded a χ^2 rather small compared with the number of degrees of freedom The errors in the daily-average arrival times are apparently overestimated in these cases, perhaps as a result of 'pulse jitter' predominantly on time scales of several minutes The two pulsars PSR0611+23 and PP0943 behave in the opposite way, having a much larger value of χ^2 than expected It may well be that these two objects are further examples of pulsars exhibiting erratic behaviour in pulse phase on time scales greater than a few minutes, as do the Crab pulsar[5], PSR1642—03 and PSR0329+54 (ref, 17) Another possible explanation is in terms of the changes in pulse shape that are seen in averages of several minutes for both

Table 1 Information on pulsars for which timing results were obtained by least squares

Serial no	Discovery name	Ref	Interval fitted (day/month/ year)	Degrees of freedom	χ^2	R M S Residual (μs)	$\tau = P/(dP/dt)$ (10^6yr)
1	PSR0301+19	6, 7	14/08/72–06/04/74	16	7 2	766	34
2	PSR0540+23	8, 9	26/01/73–07/03/74	11	2 0	146	0 51
3	PSR0611+22	8, 9	26/01/73–08/03/74	12	43 3	643	0 18
4	PP0943	10, 11	14/12/72–05/04/74	12	33 6	4,887	9 9
5	JP1858	11, 12	02/06/72–12/03/74	20	11 7	1,387	2 8
6	PSR1907+02	8	26/03/73–23/03/74	11	5 4	751	5 7
7	OP1914+13	7, 13	05/09/72–16/01/74	13	20 1	241	0 86
8	PSR1917+00	8	26/03/73–23/03/74	11	3 5	927	5 3
9	MP1944	7, 14	02/06/72–23/03/74	21	16 7	582	570
10	JP1946	11, 15	03/07/72–12/03/74	13	14 7	507	3 2
11	JP1953	11, 12	16/02/73–12/03/74	11	6 9	1,260	3,980 (1,290*)
12	JP2003	7, 12	03/07/72–12/03/74	16	4 9	558	0 90
13	PSR2020+29	7, 16	13/12/72–23/03/74	13	4 4	222	5 7

*Corresponds to maximum dP/dt allowed by error bar

Table 2 Fitted parameters for thirteen pulsars*

Serial no	RA (1950) h min s	Dec	Epoch (JD–2440000)	Period (A 1s)	dP/dt (10^{-15}s s^{-1})
1	03 01 42 43 0 05	19°21′11 6 2 2	1543 8750	1 387 583 579 433 18	1 294 20 58
2	05 40 06 86 0 09	23 28 15 4 33 8	1708 5625	0 245 965 194 092 10	15 428 78 59
3	06 11 15 59 0 05	22 31 42 0 20 0	1708 6250	0 334 919 019 183 72	59 731 0 4 6
4	09 43 27 24 0 41	10 05 50 0 16 4	1665 8125	1 097 703 639 72 94	3 533 43
5	18 59 01 923 0 014	03 26 46 3 0 6	1470 7500	0 655 444 707 516 46	7 502 0 1 8
6	19 07 07 75 0 05	02 49 55 8 0 6	1767 9375	0 494 913 709 26 24	2 762 14
7	19 15 21 566 0 004	13 48 28 5 0 1	1565 5000	0 194 625 883 200 7	7 201 57 27
8	19 17 17 22 0 01	00 16 03 0 0 3	1767 9375	1 272 255 295 54 13	7 674 8
9	19 44 38 75 0 01	17 58 15 4 0 2	1470 8125	0 440 618 461 656 14	0 0244 5
10	19 46 33 945 0 007	35 32 38 2 0 2	1501 6875	0 717 306 326 897 50	7 054 7 1 4
11	19 52 21 83 0 03	29 15 22 3 0 5	1730 0625	0 426 676 785 45 11	0 003 4 7 1
12	20 02 53 715 0 006	31 28 34 0 0 1	1501 7500	2 111 211 782 83 6	74 581 8 1 8
13	20 20 33 295 0 004	28 44 43 2 0 1	1665 3125	0 343 400 843 442 9	1 895 6 5

*Errors in least significant digits are shown below each parameter

PSR0611+23 and PP0943 A quantitative study of this effect has not yet been made

Perhaps the most surprising results of these measurements are the unusually small values of dP/dt for MP1944 and JP1953, leading to the long 'lifetimes' of Table 1 According to our tabulation of dP/dt for 42 pulsars, dP/dt for MP1944 is about one order of magnitude less than the previous smallest value (AP2016—$dP/dt = 0\ 15 \times 10^{-15}$), and that for JP1953 may be two orders of magnitude smaller F D Drake (personal cummunication) has suggested that JP1953 may in fact be a white dwarf rather than a neutron star, but at present there seems to be no definitive way to confirm this possibility by calculation The total radio flux, an essential parameter in testing such a hypothesis, has, unfortunately, not yet been measured for either JP1953 or MP1944 Until more is known about the emission mechanism of pulsars, it will be difficult to decide on theoretical grounds whether or not JP1953 represents a new class of object

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Effects of thermal photon scattering on relativistic electrons near pulsars

WE assume that pulsar radiation is generated close to the surface of a neutron star The production of this radiation clearly involves some sort of coherent motion of relativistic electrons, which are presumed to have been accelerated off the stellar surface by large electric fields along magnetic field lines of force Rengarajan[1] has pointed out that these electrons may scatter against black body photons emitted by the hot neutron star The hotter the star, the greater is the density of these photons, and the greater the probability that electrons will scatter against them Our purpose here is to examine the consequences of such collisions and the conditions required for them to take place

First, we find the limit on the neutron star temperature such that scattering occurs between a thermal photon and a relativistic electron Let $\mathscr{E}'$ be the energy of a photon in the rest frame of the electron, and $\mathscr{E}$ the corresponding energy in the rest frame of the neutron star We have $\mathscr{E}' \simeq \gamma\mathscr{E}$ and $\mathscr{E} \simeq 3kT$ where γ is the Lorentz factor of the electron, k is Boltzmann's constant and T is the surface temperature of the star Defining

$$\chi \equiv (2\mathscr{E}'/mc^2) \simeq (6\gamma kT/mc^2) \qquad (1)$$

(where m is the mass of the electron and c the speed of light), the cross section σ for electron-photon scattering is given by the Klein–Nishina formula[2]

$$\sigma = \begin{cases} \sigma_T & \text{for } \chi \ll 2 \qquad (2a) \\ 3/4\,\sigma_T \dfrac{(ln\chi+1/2)}{\chi} & \text{for } \chi \gg 2 \qquad (2b) \end{cases}$$

where the Thompson cross section $\sigma_T = 0\ 66\times 10^{-24}$ cm^2 The scattering cross section is Thompson if $\chi \ll 2$, $\chi = 2$ corresponds to $\gamma = \gamma_T \equiv 2\times 10^9\ T^{-1}$ If $\gamma \gg \gamma_T$, the scattering is Compton The number density of photons within a black body enclosure is 0 244 $(kT/hc)^3$, where h is Planck's constant, n, their number density just above the neutron star surface, is half this value The mean free path λ of an electron is then $\lambda \simeq (n\sigma)^{-1}$ for distances much less than the stellar radius

Regarajan estimates the critical temperature in order that mildly relativistic electrons scatter within about one stellar radius R from the surface With $\gamma \leqslant 10^3$ the scattering is Thompson or close to it, so that the condition that $\lambda \leqslant R$ is approximately given by $R \geqslant (n\sigma_T)^{-1}$ Thus he finds (using a slightly more rigorous method)

$$T \gtrsim R^{-1/3}\ 10^8,$$

which for $R = 10^6$ cm yields the limit $T \geqslant 10^6$ K, a very reasonable temperature for pulsars We will show, however, that such estimates are good only if the accelerating electric field strengths are unusually low

We now consider an electron accelerated by an electric field along a magnetic field line leading away from the surface of a neutron star If it scatters (against anything) and acquires a component of momentum across the magnetic field, it is then decelerated by the emission of synchrotron radiation The energy of such an electron in a medium with aligned electric (E) and magnetic (B) fields has an energy in time[3]

$$\gamma(t)mc^2 = mc^2[1-\beta_\perp^2(1-\beta_\parallel^2)^{-1}\exp(-2\Omega_B\delta s)]^{-1/2} \cosh(\Omega_B\alpha s+a) \qquad (3)$$

where s is the proper time given by $t = \int_0^s[\gamma(s')]^{-1}ds'$, and $\Omega_B = eB/mc$, $\alpha = E/B$, $\delta = \Omega_B\tau(1+\alpha^2)$, and $\tau = 3/2\ e^2/mc^3$ Here $\cosh a = (1-\beta^2)^{-1/2}$, where $c\beta_\parallel$ and $c\beta_\perp$ are the initial velocities parallel and perpendicular to the field For pulsar parameters $B = 10^{12}$ and $E \ll B$ we have $1 \gg \delta \gg \alpha$, the electron energy drops rapidly to $\mathscr{E}_{\min} = mc^2(1-\beta_\parallel^2)^{-1/2}$ in a proper time $s \sim (\Omega_B\delta)^{-1}$, and then begins to accelerate parallel to the field

Suppose first that the scattering is Thompson In Thompson scattering the energy of the photon is typically increased to $6\gamma^2kT$ This photon is emitted at an angle $\theta \sim \gamma^{-1}$ to the initial direction of the electron along the magnetic field The change in perpendicular momentum of the photon is then about $6\theta\gamma^2kT/c$ or $6\gamma kT/c$ The electron likewise experiences a change of perpendicular momentum $p_\perp \approx 6\gamma kT/c$ and its $\beta_\perp \approx 6kT/mc^2$ This momentum is non-relativistic so that the electron will emit cyclotron radiation at frequency[4] $\gamma\Omega_B$ From equation (3) we see that it will lose primarily only its perpendicular energy, which is small compared with its total energy, before being reaccelerated So the electron's motion along the magnetic field line is essentially unaffected by Thompson scattering (This discussion corrects that of Rengarajan[1], who erroneously concluded that Thompson scattering would drastically affect the electron's motion)

Secondly suppose that the scattering is Compton In Compton scattering the scattered photon and electron typically come out with about the same energy ($\sim 1/2\ \gamma mc^2$) at an angle $\theta \approx (6kT/\gamma mc^2)^{1/2}$ The electron's initial perpendicular momentum is about $p_\perp = (3/2\ \gamma kT/mc^2)^{1/2}mc$ and is relativistic ($\gg mc$) From equation (3) we see that its final energy before being reaccelerated, though relativistic, is now much less than its initial energy

$$1 \ll \gamma_{\text{final}} \simeq (\gamma mc^2/6kT)^{1/2}$$

For example, with $\gamma = 10^7$ and $T = 10^6$, $\gamma_{\text{final}} \simeq 10^{-2}\gamma$ Because the cross section for scattering (equation (2)) increases as γ decreases, the probability for scattering will again be greatly increased In fact the electron will Compton scatter again before it can recover its initial energy In this way the electron's energy will be progressively decreased until $\chi \simeq 2$, after which the scatterings are in the Thompson regime and the electron's energy will be only slightly affected by collisions

Compton scatterings will therefore limit electron energies in pulsar magnetospheres to $\gamma_T = 2\times 10^9T^{-1}$ Many people regard the radio emission from pulsars as curvature radiation In order for this to be so one requires $\gamma \geq 10^3$, which places an upper limit on the temperatures of the pulsars of about 2×10^6 K This limit, however, is only relevant if such scatterings do, in fact, take place This, in turn, is a question which depends on the specific structure of the pulsar magnetosphere, and we have been unable to come to any general conclusions with regard to it In what follows we will re-examine the conditions for scattering within the context of the pulsar model proposed by Sturrock[5]

Within Sturrock's model the electric field E increases linearly with distance r above the surface and is given by $E=3\times 10^2\,rP^{-1}$ statvolt cm^{-1} for $r \leq r_P \equiv 2\times 10^4\ P^{-1/2}$, where P is the pulsar's rotation period in seconds If the electron accelerates from rest at the surface it will have an energy $\gamma mc^2 = 1/2\ Eer$ at a height r, or $\gamma = \gamma_r = 10^{-1}r^2P^{-1}$ But in fast pulsars with $P \leqslant 0\ 7$ s, at a distance of $r_L = 3\times 10^4\ P^{4/7}$, the emission of curvature radiation slows the increase of energy, limiting γ to $\gamma_L = 10^7\ r^{1/4}$ for $r_L \leq r \leq r_P$ Finally, at heights $r \geq r_P$ the accelerating electric field presumably decreases with a scale height of about r_P The energy of the electron then rapidly decreases due to the continual emission of curvature radiation At about one stellar radius the energy reaches its asymptotic value given by $\gamma \simeq \gamma_\infty = 6\times10^7P^{1/3}$ for fast pulsars and $\gamma \simeq \gamma_{\max} = 3\times10^7P^{-2}$ for slow pulsars ($P \geqslant 0\ 7$ s)

The scattering cross section will be Thompson if $\gamma \leq \gamma_T$, that is at heights $r < r_T \equiv 2\times10^5T^{-1/2}P^{1/2}$ The condition that the mean free path $\lambda_T = (n\sigma_T)^{-1} \approx 10^{23}T^{-3}$ by less than r_T requires $T > T'$ where $T' = 2\times10^7P^{1/5}$ K This condition is more severe than that given by Rengarajan, because he required only that $\lambda \leqslant R \simeq 10^6$ cm Turning then to the Compton scattering limit $\chi \gg 2$ we have the mean free path

$$\lambda = 4/3\,\lambda_T[\chi/(ln\chi+1/2)] \qquad (4)$$

and $\chi = 6\times10^{-11}\ Tr^2P^{-1}$ for $r_T \leq r \leq r_L$ At $r = r_T$, $\lambda > r$, because $\chi \propto r^2$, λ increases faster than r Thus $\lambda \gg r$ for $r > r_T$ and electrons will never scatter in this regime For $r_L \leq r \leq r_P$ in fast pulsars the energy of the electron is ultimately radiation reaction limited, and $\chi = 10^{-2}Tr^{1/4}$ The mean free path then increases more slowly than r, but because $\gamma \gtrsim 10^8$ already, $\lambda \leqslant r_P$ only if $T \geqslant 10^8$ K Finally, beyond r_P, γ decreases and the scattering cross section increases The condition that $\lambda \leqslant R$ at $r = R$, where R is the stellar radius, is that

$$T^2 \ln(10^{-0\ 8}P^{1/2}T) \geq 10^{16}P^{1/3}$$

for fast pulsars and that

$$T^2 \ln(10^{-1\ 3}P^{-2}T) \geqslant 10^{15\ 6}P^{-2}$$

for slow pulsars Thus electrons will Compton scatter at least once if T exceeds $10^{7\pm0\ 2}$ K for $3\times10^{-3} \leqslant P \leqslant 3$ s

These limits for both Compton and Thompson scattering lie significantly above the observational upper limits on the temperatures of the known pulsars[6] The processes under consideration cannot, therefore, be occurring within them With regard to new pulsars, however, the situation is reversed, for the surface temperature of a newly-formed neutron star is very great By our analysis such a neutron star will be unable to generate curvature radiation (because of the low limits on γ) until it has cooled to below 10^7 K, a process which may take as long as several years Thus we conclude that very young neutron stars may not be pulsars

The above model conditions for scattering are, of course, dependent on the specific spacial structure and strengths of the electric fields that give rise to the radiating electrons The electric fields may in fact be much weaker than those chosen, thus less stringent (lower) limits on the temperatures are possible But in stars of low temperatures the photon-electron scatterings will have negligible effect on the coherence properties of the electron stream Therefore, it is doubtful that thermal photon collisions with the radiating electrons place any theoretical limits on the temperature of known pulsars

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Dicarboxylic acids in the Murchison meteorite

INVESTIGATIONS of the extractable organic material found in the Murchison meteorite, a carbonaceous chondrite, have led to the identification of many different classes of compounds, including amino acids[1–5], hydrocarbons[3,6 7], heterocycles which contain nitrogen[8 9], and monocarboxylic acids[10] We report here a large suite of aliphatic dicarboxylic acids found in extracts from the chondrite This suite includes many of the branched and straight chained isomers with up to nine carbon atoms The large number of compounds and the isomeric distribution suggest that, like the amino acids, they are the product of an abiotic synthesis

The meteorite sample used for this study was prepared by removing the outer surface and reducing the interior piece to a fine powder with a mortar and pestle A hot water extract of a 2-g sample was obtained by refluxing for 20 h with 6 ml of water After centrifuging, the supernatant and subsequent wash solution were dried by rotary evaporation and redissolved in 0 5 ml of 0 09N HCl–methanol (The 0 09 N HCl–methanol solution was prepared by dissolving 0 36 ml of 12 N HCl in 48 ml of absolute methanol) The resulting solution was applied to a bed of clean, dry silica gel (0 9 cm by 10 cm) and eluted with 0 09 N HCl–methanol The first 3 0 ml of the eluate was collected, evaporated to dryness, and the residue taken up in 3 0 ml of 1 0 NCHl saturated with sodium chloride The solution was extracted continuously with diethyl ether for 18 h The ether extract was divided into three fractions The first fraction was evaporated to dryness and the di-(+)-2-butyl esters prepared by heating the residue for 1 h at 105° C with 1 ml of absolute (+)-2-butanol which was 8 N in HCl (ref 11) The second fraction was evaporated to dryness and the *n*-butyl esters prepared by heating the dry residue for 30 min at reflux with 1 ml of absolute *n*-butanol which was 1 25 N in HCl (ref 12) Similarly, the methyl esters were prepared by reacting the residue of the dry third fraction in methanol, with ethereal diazomethane

The di-(+)-2-butyl esters were separated by gas chromatography (GC) using a wall coated column of UCON 75 H-90000 (150 feet by 0 02 inch) The column was programmed from 100° C to 155° C at 1°C min^{-1} The same gas chromatograph was coupled to a mass spectrometer (MS) by means of a membrane separator, for GC/MS investigations A data system was used to acquire, store, and process the data The GC and GC/MS studies of the *n*-butyl esters and methyl esters were performed using a gas

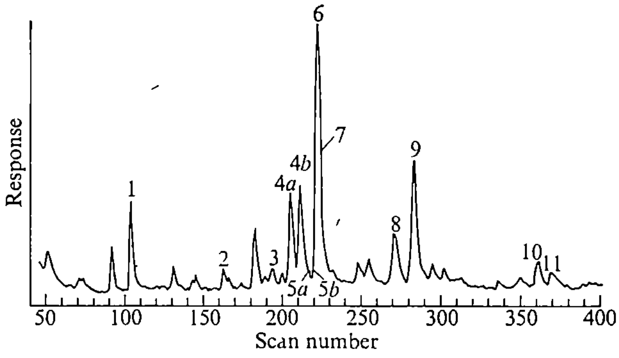

Fig. 1 Total ion plot, reconstructed by computer, of the di (+) 2-butyl esters of the dicarboxylic acids from the Murchison carbonaceous chondrite 1, Oxalic acid, 2, malonic acid, 3, 2,2-dimethylsuccinic acid, 4*a*, *b*, methylsuccinic acid, 5*a*, *b*, 2,3-dimethylsuccinic acid, 6, succinic acid, 7, fumaric and/or maleic acid, 8, 3-methylglutaric acid, 9, glutaric acid, 10, adipic acid, 11,3-methyladipic acid

chromatograph coupled by means of an all-glass membrane separator[13] to a mass spectrometer interfaced to a computer system The GC separations were carried out using a coiled-glass column (6 feet by 0 25 inch) packed with Tabsorb The column was programmed from 100° C to 210° C at 4° C $\min^{-1}$ Both mass spectrometers were operated at an ionising energy of 70 eV A procedural blank showed no dicarboxylic acids

The dicarboxylic acids found in the extract of the chondrite have been identified by their GC rentention values and by comparison of their mass spectra with those of authentic standards Thus, 17 dicarboxylic acids have been identified positively (Table 1) In addition, numerous other GC peaks were observed (Figs 1 and 2), many of which yielded mass spectra suggestive of the aliphatic dicarboxylic acids, but which, at this time, remain unidentified because of a lack of authentic standards

The presence of a large suite of dicarboxylic acids in carbonaceous chondrites has not been reported previously The isolated dicarboxylic acids consisted of a number of straight and branched chain isomers with up to nine carbon atoms All of the structural isomers of the dicarboxylic acids with two, three and four carbon atoms have been identified, and six of the eight possible structural isomers of the aliphatic dicarboxylic acids with five and six carbon atoms have been identified Many of the remaining isomers seem to be present, that must be confirmed by comparison with authentic standards The presence of a large number of isomers of the low molecular weight dicarboxylic acids is analogous to the distribution of amino acids found in this specimen of the Murchison meteorite (Pereira, W E , *et al*, unpublished), it is also analogous to the distribution of amino acids and monocarboxylic acids found in other specimens of the Murchison and Murray carbonaceous chondrites[1-5,10] The distribution of the aliphatic dicarboxylic acids suggests, therefore, that a random chemical synthesis may be responsible for their presence The state of all of the dicarboxylic acids in the meteorite sample is not known Whewellite, the calcium salt of oxalic acid has, however, been isolated and identified[14] Whether all of these compounds occur as salts or whether they are present as free dicarboxylic acids or some type of precursor to the dicarboxylic acids is unknown

Table 1 Dicarboxylic acids found in extracts of the Murchison meteorite

Oxalic acid
Malonic acid
2,2-Dimethylsuccinic acid
Methylsuccinic acid
2,3 Dimethylsuccinic acid
Succinic acid
Maleic and/or fumaric acid
3-Methylglutaric acid
Glutaric acid
Adipic acid
3-Methyladipic acid
Pimelic acid
Suberic acid
Azelaic acid
Methylmalonic acid
3,3-Dimethylglutaric acid
2,2-Dimethylglutaric acid

In addition to the saturated dicarboxylic acids, some unsaturated dicarboxylic acids were observed Mass chromatography has been used to detect an unsaturated dicarboxylic acid (maleic and/or fumaric acid) which elutes with succinic acid during GC analysis But maleic and fumaric acids have the same GC retention values on the columns which we used, and their mass spectra are virtually identical, so we were unable to distinguish between these two dicarboxylic acids in this investigation

In addition to the large number of structural isomers found in this sample, some of the dicarboxylic acids with asymmetrical centres show a GC separation of their enantiomers (see Fig 1) Although the separation of the optical isomers of amino acids has been accomplished by the preparation of the diastereoisomeric (+)-2-butyl derivatives, similar separation of the optical isomers of the dicarboxylic acids as the (+)-2-butyl diesters has not been reported previously A detailed investigation of the appropriate conditions, reagents, GC columns, and so on, necessary to derive these products, is in progress and will be reported in the future It should be noted that, in the manner analogous to amino acids obtained from abiotic sources, both enantiomers of R and S-methyl succinic acid seem to be present in nearly equal concentration (see Fig 1) A similar finding has been reported for a stimulated, abiotic, chemical evolution experiment[15] The continued investigation of the dicarboxylic acids is important because, apart from amino acids, they are the only other compounds so far found in meteorites in which the optical isomers have been separated The aliphatic dicarboxylic acids thus represent a potential suite of marker compounds for distinguishing between indigenous nonbiological and biological compounds found in extraterrestrial samples In addition, they are present in extracts of the Murchison meteorite at concentrations approximately one to two orders of magnitude higher than the amino acids The exact quantity and distribution of aliphatic dicarboxylic acids, when compared with similar information for amino acids and fatty acids, should provide insight into the organic reactions responsible for their presence in carbonaceous chondrites

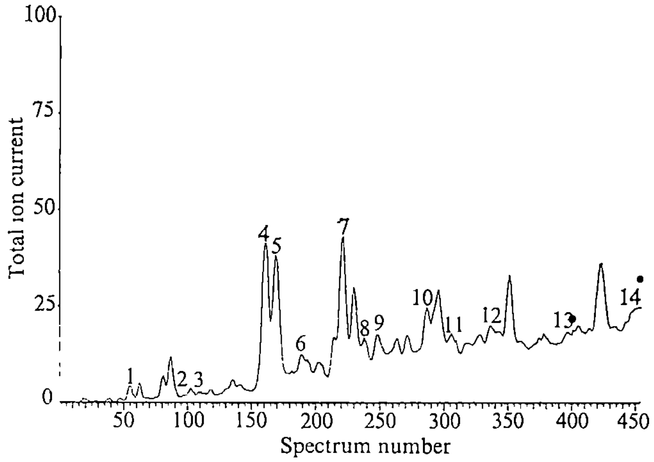

Fig 2 Total ion plot, reconstructed by computer, of the di-*n* butyl esters of the dicarboxylic acids extracted from the Murchison carbonaceous chondrite 1, Oxalic acid, 2, methylmalonic acid, 3, malonic acid, 4, succinic and maleic or fumaric acid, 5, methylsuccinic acid, 6, 2,3-dimethylsuccinic acid, 7, glutaric acid, 8, 3-methylglutaric acid, and 3,3-dimethylglutaric acid, 9, 2,2-dimethylglutaric acid, 10, adipic acid, 11, 3-methyladipic acid, 12, pimelic acid, 13, suberic acid, 14, azelaic acid

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Dicarboxylic acids from electric discharge

OPARIN[1] and Urey[2] have suggested that methane, ammonia, hydrogen and water were present in the primitive atmosphere of the Earth Significant yields of amino, hydroxy, and monocarboxylic acids have been produced by the action of electric discharge on such a primordial gas mixture[3,4], and numerous chemical evolution experiments have produced many types of biologically significant molecules[5] Apart from the detection of succinic acid[3,4,6], no other dicarboxylic acids have been reported in chemical evolution experiments

A large suite of dicarboxylic acids, indigenous to the Murchison meteorite, have been identified, and they were probably produced in a random, nonbiological synthesis[7] As a result of this discovery, the possible synthesis of a similar suite of dicarboxylic acids was investigated in a chemical evolution experiment which simulated electric discharge through the primitive atmosphere of the Earth Among the products of this experiment, saturated, unsaturated, methyl and chloro-substituted dicarboxylic acids were detected, using gas chromatography (GC) and mass spectrometry (MS)

A 10-l electric discharge chamber equipped with four Tesla coils was used (see ref 8) A reaction mixture of 200 mm CH_4, 200 mm N_2, and 400 ml NH_4Cl solution (0 05 M, *p*H 8 77) was sparked for 48 h (ref 9) The aqueous phase was rendered strongly basic with NH_4OH, then filtered and stored in a freezer until the analysis Fifteen millilitres of this filtrate was concentrated to 5 ml by freeze-drying For the analysis, 0 5 ml of the concentrated filtrate was evaporated to dryness and redissolved in 0 5 ml of 0 09 N HCl in methanol (prepared by adding 0.36 ml 12 N HCl to 48 ml of absolute methanol) This solution was applied to a dry column (0 9 cm by 10 cm) of Adsorbosil-2, a silica gel without binder This adsorbent was batch-washed seven times with 0 1 N HCl in methanol, then dried and equilibrated to room humidity before use After the application of the sample, a 10-cm head of 0 09 N HCl in methanol was placed over the column and the chromatography was begun When the solvent reached the bottom of the column, nitrogen pressure was applied and fractions of 0.5 ml were collected The fractions were monitored using thin-layer chromatography The first 2 5 ml (containing the amino acids and dicarboxylic acids) were centrifuged to remove silica gel fines and then evaporated to dryness The residue was dissolved in 1 N HCl saturated with NaCl This solution was continuously extracted with diethyl ether for 16 h (to separate the amino acids from the dicarboxylic acids) The ether extract was dried, and diester derivatives of the dicarboxylic acids were prepared by heating the residue for 1 h, under anhydrous conditions, with (+)–2–butanol which was 5 N in HCl. Excess reagent was evaporated with a stream of dry, filtered nitrogen The di–(+)–2–butyl esters of the diacid were dissolved in 10 μl of methylene chloride A 2-μl sample of this solution (equivalent to 0 25 ml of the original electric discharge aqueous phase) was subjected to analysis by GC/MS A gas chromatograph was coupled to a mass spectrometer (operated at 70 eV) by means of a membrane separator A data system was used to acquire, store, and process the data No dicarboxylic acids were detected in the procedural blank Identification of the derivatised diacid electric discharge products is based on laboratory comparisons of their mass spectra with those of authentic standards Further confirmation was obtained by comparing gas chromatographic retention times of the products and standard compounds by co-injection techniques No significant racemisation was observed when R-methylsuccinic acid was run through the analytical procedure

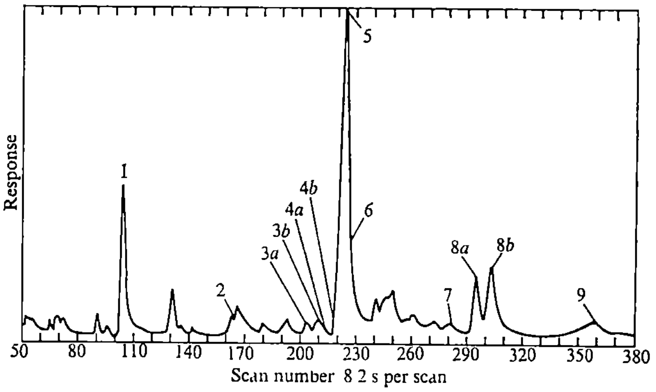

Fig 1 Gas chromatogram, reconstructed by computer, of the di–(+)–2–butyl esters of the dicarboxylic acid products of electrical discharge Identifying numbers as in Table 1 A wall coated column of UCON 75 H 90000 (150 feet by 0 02 inches) was temperature programmed from 100° C to 150° C at 1° C min^{-1} The helium flow was 5 ml min^{-1}.

Figure 1 shows the gas chromatographic separation of the derivatives of the diacids produced by spark discharge Table 1 lists those compounds that were confirmed by correlation of both chromatographic retention times and mass spectra with the corresponding authentic standards Mass chromatography was used to detect fumaric and/or maleic acid which elute with succinic acid The characteristic *m/e* 157 for succinic acid and *m/e* 155 for fumaric and maleic acid were monitored (Fig 2) These mass fragments correspond to the fragments of the respective derivatives minus one butoxy group Fumaric and maleic acid have identical retention times under the chromatographic conditions used and their mass spectra are virtually the same In a similar manner, mass chromatography revealed two peaks for the three possible configurational isomers of 2,3–dimethylsuccinic acid The use of optically active (+)–2–butanol to prepare diester derivatives of the diacids was necessary for the separation of those compounds containing asymmetric carbon atoms Enantiomeric pairs, such as R,S–methylsuccinic acid and R,S–2–chlorosuccinic acid, were separated using gas chromatography, with good resolution The exact order of elution of the enantiomers is under investigation in our laboratory, subject to the availability of pure, optically active standards

Table 1 Dicarboxylic acids identified as products of electric discharge

1	Oxalic acid	5	Succinic acid
2	Malonic acid	6	Fumaric and/or maleic acid
3*a*	Methylsuccinic acid*	7	Glutaric acid
3*b*	Methylsuccinic acid*	8*a*	2-Chlorosuccinic acid*
4*a*	2,3-Dimethylsuccinic acid*	8*b*	2-Chlorosuccinic acid*
4*b*	2,3-Dimethylsuccinic acid*	9	Adipic acid

* Elution order of configurational isomers is uncertain

Five of the eight possible structural isomers of the nonchlorine containing aliphatic dicarboxylic acids with two to five carbon atoms have been detected Furthermore, many of the unidentified peaks (Fig 1) have mass spectra suggestive of dicarboxylic acids Their identification is subject to the availability of pure standards The variety of structural isomers found is not surprising, because similar arrays of isomers arising from random chemical synthesis have been detected for hydrocarbons, amino acids, and monocarboxylic acids derived from chemical evolution studies[5]

The suite of dicarboxylic acids in the electric discharge experiment is similar to that of the Murchison meteorite, except for the fact that 2–chlorosuccinic acid is present in the spark discharge Because NH_4Cl was used in the experiment, chloro-acids were probably formed by the interaction of the discharge with aerosol particles suspended in the gas phase, or by interaction of chloride ions with reactive intermediates in the liquid phase, or both

Apart from work with amino acids[9], this gas chromatographic separation of R,S isomers represents the only case in which molecules possessing asymmetric carbon atoms synthesised in chemical evolution experiments were resolved chromatographically This gas chromatographic capability seems to provide a rapid, routine, new tool for the differentiation of biologically and nonbiologically produced dicarboxylic acids

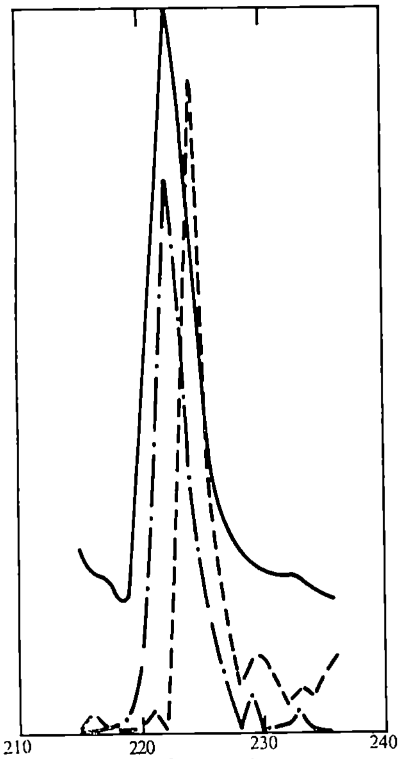

Fig. 2 Mass chromatogram, reconstructed by computer, of a portion of the GC/MS run shown on Fig 1 Solid curve, total ion current (scaling factor=1), dashed curve, $m/e=155$ (scaling factor=128), — — —, $m/e=157$ (scaling factor=16)

Dicarboxylic acids are important metabolic intermediates, especially with respect to photosynthesis and bioenergetics (as in Krebs cycle) The discovery that these compounds can be produced by spark discharge supports the view that dicarboxylic acids were probably present in the prebiotic *milieu* Thus, the abiotic synthesis of this class of molecules has implications for chemical evolution Because similar arrays of dicarboxylic acids, as well as amino acids[9,10], were found in the Murchison meteorite and the electric discharge experiments, it is possible that a high energy process such as electric discharge, played an important role in the production of the organic matter found in meteorites To explore this relationship, we are engaged in comparative studies of the organic and stable isotope chemistry of the meteoritic and synthetic products Such studies may shed light on the conditions of, and mechanisms involved in, organic synthesis in the early Solar System

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Vertical mixing in the deep ocean

SEVERAL authors[1-3] have reported *in situ* observations of light scattering intensity in the deep ocean, which reveal the widespread occurrence of an ~ 1 km thick layer of suspended mineral and organic matter above the ocean floor The thickness of this nepheloid layer is several orders of magnitude greater than the characteristic thickness of the turbulent Ekman layer at the ocean floor[4] Analysis of observations of the nepheloid layer in the western North Atlantic indicates that an average vertical mixing coefficient, A, ~ 100 cm² s⁻¹ is required to maintain the layer[2] in the lowest kilometre or so of the ocean This value is significantly greater than the traditional (1 cm² s⁻¹) estimate based on water mass analysis[5,6] of vertical eddy diffusivity in the deep ocean

Here, I point out that this rather large mixing coefficient is consistent with mixing induced by internal waves[6,7] in the lowest kilometre or so of the ocean The relatively high vertical mixing rates in that region can be accounted for by the peculiarities of the generation of internal waves as a result of the interaction of topographic Rossby waves with relatively small scale ($\leqslant 1$ km) topographic features on the ocean floor

Following Bell[8], the rate of energy production in internal waves, E, arising from the flow of a steady uniform current, of speed, U, over a rough bottom, may be approximated as

$$E \sim 2\ \rho\ fNUH^2$$

where ρ is seawater density, f is the Coriolis parameter, N is the representative Brunt–Vaisala frequency, and H is the contribution to the r m s elevation of the topography in the internal wave passband of wave numbers, k, such that $f < kU < N$ In fact, deep ocean currents are not steady, being composed for the most part of tidal and inertial period motions and quasigeostrophic motions with periods of a week or longer[9] It is this latter component of flow which is relevant here The characteristic time scale for such motions is sufficiently large that they may be considered as steady as far as processes of internal wave generation are concerned

Swallow[10] has reported the results of a series of current measurements made during a period of 14 months in the western North Atlantic. The observed structure of the currents may be interpreted in terms of bottom trapped topographic Rossby wave modes[11], with velocity decaying on a vertical scale of several kilometres, from a near bottom maximum of roughly 10 cm s^{-1}. The presence of significant topographic structuring on horizontal scales of 10 km and smaller[12] should introduce further variability in the basic current field in the lowest kilometre. The interaction of this topographically modified quasisteady current field with the smaller scale topographical features is responsible for the generation of internal wave energy. As the internal waves propagate away from the bottom, the relative frequency, kU, decays with height. That has serious implications as far as the internal wave field is concerned. The ultimate catastrophe for any given wave component occurs when it encounters a critical level[13,14] at which the relative frequency is reduced to the local inertial frequency, f. Nonlinear effects inevitably destroy the internal wave component in the vicinity of such a critical level, so that as a consequence of the general decay of background current speed with distance above the bottom, a substantial portion of the internal wave energy should be dissipated in the lowest kilometre or so of the ocean.

The rate of production of internal wave energy may be estimated for conditions representative of the western North Atlantic. A typical deep current speed is, $U \sim 10$ cm s^{-1}. A representative value for the Brunt–Vaisala frequency is[15] $N \sim 7 \times 10^{-4}$ s^{-1}. Analysis of a large number of topographical profiles of abyssal hills in the eastern North Pacific[8] suggests that a value of $H^2 \sim 10^3$ m^2 is representative, and consistent with the results of an analysis of sonic sections[16] in the North Atlantic. At a latitude of 30° N ($f \sim 7 \times 10^{-5}$ s^{-1}), then, $E \sim 10$ erg cm^{-2} s^{-1}. If it is assumed that 50% of that energy goes into mixing in the lowest kilometre of the ocean, then there must be an associated buoyancy flux, F, $\sim 5 \times 10^{-5}$ cm^2 s^{-3}. With a Brunt–Vaisala frequency, N, $\sim 7 \times 10^{-4}$ s^{-1}, that implies a vertical mixing coefficient ($A = F/N^2$) of ~ 100 cm^2 s^{-1}. That is precisely of the same order as the vertical mixing coefficient based on the analysis of observations of the western North Atlantic nepheloid layer[2]. Further support of the proposed relationship between mixing induced by internal waves and the nepheloid layer is provided by the apparent correlation between bottom roughness and thickness of the nepheloid layer[3]. The mixing induced by internal waves discussed here must be balanced in the overall thermohaline energy budget. Acting over 50% of the North Atlantic, the vertical mixing estimate made here implies a rate of energy conversion of the order of 10^{18} erg s^{-1}. In this connection, Wright[17] has investigated the rate at which available potential energy is released by the sinking of cold water masses in the northern North Atlantic. He found that the two Norwegian Sea overflows, and winter convection in the Labrador Sea, contributed $\sim 0.85 \times 10^{18}$ erg s^{-1}. The North Atlantic seems to be somewhat unique in the intensity of its deep currents. Near-bottom currents of 3 or 4 cm s^{-1} are probably more representative of the world ocean[4], so that an estimate of E, ~ 1 erg cm^{-2} s^{-1}, with a corresponding mixing coefficient, A, of the order of 10 cm^2 s^{-1} may be considered as more representative of the world ocean.

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Thermonuclear micro-explosions with intense ion beams

HIGH density pellet compression may be important in laser fusion[1]. In this way the minimum energy input for break-even can be substantially reduced to levels within reach of expected future laser technology. Further[2] when this concept is applied to a fissionable pellet surrounded by a small neutron reflector, preferably TD, which is compressed together with the reflector, to high densities, very small critical masses become possible, in addition the fission process could ignite a thermonuclear reaction in the TD reflector. In these proposals very large laser energies of ~ 1 MJ, which may be difficult to reach, are required. As an alternative to laser fusion I have proposed the use of intense relativistic electron beams[3], but here the beam pulse length is longer than for laser beams, and the electron stopping range may be too long.

I present here an alternative idea based on the possibility of generating intense ion beams in a magnetically insulated diode[4,5]. In such a diode a pulsed laser beam is directed on to the anode surface as a high voltage is applied to the diode, and a magnetic field directed parallel to the cathode surface prevents the flow of electrons from the cathode to the anode, but not the flow of ions from the anode to the cathode, provided that the magnetic field is strong enough to make the electron Larmor radius, but not the ion Larmor radius, small compared with the anode–cathode gap. The laser beam hitting the anode surface will produce a surface plasma from which the ions are drawn and accelerated towards the cathode. After their passage through the cathode, which has the form of a grid to minimise the energy losses by collision, the ions are projected into a drift tube. This drift tube is filled with a tenuous plasma of high electrical conductivity. Because of their large inertia the ions draw into their flow electrons which pass through the highly conducting plasma from the cathode surface or the inner surface of the drift tube. They thus compensate for an electric space charge field which would otherwise build up. Furthermore, because of their small mass and high mobility, the electrons will follow the ion flow with the same velocity, thus also cancelling the self-magnetic field of the ion beam entering the drift tube. The ion beam therefore drifts freely as an electrically and magnetically neutral beam.

Calculations for magnetically insulated diodes pulsed with several megavolt predict current densities of the order $j \sim 3$ kA cm^{-2} such that for an area of ~ 30 cm^2 a total current of $I \sim 10^5$ A could be produced[5]. To deliver a total energy of ~ 1 MJ with a voltage of $V \sim 10^7$ V and current of $I \sim 10^5$ A the total discharge time would be $\tau \sim 10^{-6}$ s. In order to take full advantage of the low ion velocities we may choose singly

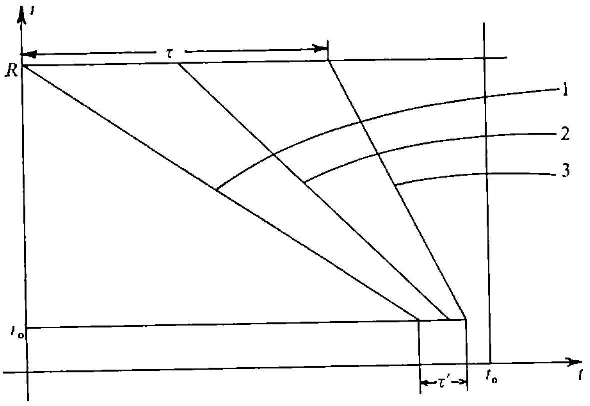

Fig 1 Time focusing of the beam

ionised heavy ions, such as ions of lead or uranium, with which the anode surface would have to be made or coated For a voltage V and atomic weight A, the ion velocity is given by $v \simeq 1\ 38 \times 10^6\sqrt{(V/A)}$ cm s^{-1} Putting $A = 200$ gives $v \simeq 10^5\sqrt{V}$ cm s^{-1} For $V = 10^6$ V, $v \simeq 10^8$ cm s^{-1} and for $V = 10^7$ V, $v \simeq 3 \times 10^8$ cm s^{-1} I propose that this variation of the ion velocity with the voltage may be used to focus the beam by increasing the voltage at the diode during the pulse Assume for example, that the pulse lasts $\simeq 0\ 7 \times 10^{-6}$ s and that an ion at the beginning of the pulse has a velocity of $\simeq 10^8$ cm s^{-1}, as compared with another ion at the end of the pulse with a velocity of $\simeq 3 \times 10^8$ cm s^{-1} One can then easily calculate that after the time $\simeq 10^{-6}$ s the ion emitted at the end of the pulse would have caught up with the ion emitted at the beginning of the pulse, at a distance of $\simeq$ 100 cm and time $\simeq 3 \times 10^{-7}$ s after the end of the pulse So by shaping the time behaviour of the voltage pulse properly at the diode one can make the ions converge at the same time and place, shortening the beam pulse and raising the beam power by perhaps many orders of magnitude

By making the ion source spherically symmetric and by projecting a beam of spherical symmetry towards a common centre of convergence, the total beam power density can be further increased This could be approximated by a large number of diodes in a spherically symmetric arrangement, with the diodes having a concave shape so that each beam pulse would have the form of a spherical segment

Figure 1 shows how the ion velocity would have to be changed in time during the pulse to focus the beam at time $t = t_0$ and at the common centre of convergence $r = 0$ The ions are emitted at the position $r = R$ The lines 1, 2 and 3 represent the $r - t$ diagram for the initial and intermediate and the final ion of the pulse Time focusing of the beam then requires for the ion velocity to follow

$$v = R/(t_0 - t) \qquad (1)$$

If a target to be bombarded by the beam is placed at $r = r_0$, the pulse length at the target τ' (at which $r = r_0$) is then shortened in comparison to the pulse length at the source (at which $r = R$) by the factor $\tau'/\tau = R/r_0$

It follows that for an ion velocity of v and a pulse length τ a linear beam can be focused in time after a distance $x \sim v\tau$, with a beam energy $E \sim IVx/v$ For $I \sim 10^5$ A, $V \sim 10^7$ V and $v \sim 10^8$ cm s^{-1} it follows that $E \sim 10^4 x$ J Therefore, for $x = 10^2$ cm, with $\tau \sim 10^{-6}$ s one would have $E \sim 1$ MJ and for $x = 10^4$ cm with $\tau \sim 10^{-4}$ s one would have $E \sim 100$ MJ A drift tube 100 m long is still well within the technical possibilities and the energy of 100 MJ to be pumped into the beam could probably be supplied by an inductive energy storage system But the energy stored in the drift tube can be delivered in a much shorter time depending on the perfection of the time focusing, which depends on the velocity spread of the ions Since the ions are produced from the anode material by a pulsed laser beam, two types of velocity spreads will occur first, by a differing degree of ionisation, second, by the initial thermal motion It is conceivable that by a properly chosen laser frequency a high degree of uniform ionisation can be achieved The most important velocity spread therefore results from the initial thermal ion energy at the anode surface This energy is of the order 1 eV In the anode–cathode gap the ions are then accelerated by a voltage of $\sim 10^6$–10^7 V, which for singly ionised ions implies an energy of $\sim 10^6$–10^7 eV Therefore, the beam will be monoenergetic to the order $\sim 10^{-6}$ to 10^{-7} corresponding to a relative velocity spread of $\sim 10^{-3}$ to $10^{-3\ 5}$ Thus, a time focusing of the beam by the same factor will be possible This would imply, for example, that in the case of the 100 MJ beam the energy could be delivered in a time of about $\sim 10^{-7}$ to 3×10^{-8} s, thus raising the beam power to 3×10^{15} W

Because of the pulse shortening by time focusing which is possible because of the low ion velocities, energy can be pumped into the beam slowly by inductive energy storage devices pulsing the diode Since the peak voltage is several million volts, this might be done with a cascade of pulse transformers

The range of heavy ions in dense matter is rather small (typically $\sim 10^{-4}$ cm), so the beam can be easily stopped if projected on to a solid piece of fusionable (or fission–fusion hybrid) material Furthermore, by properly shaping the time behaviour of the laser light impinging on the anode, the ion current and thus the beam power can be changed with time This is of importance if one wants to shape the beam pulse as has been proposed by Nuckolls *et al*[1] for adiabatic high density pellet compression with a power profile approximately given by

$$\varepsilon = \text{const}\ (1 - t/t_0)^{-15/8} \qquad (2)$$

It follows that the same power profile can be obtained with

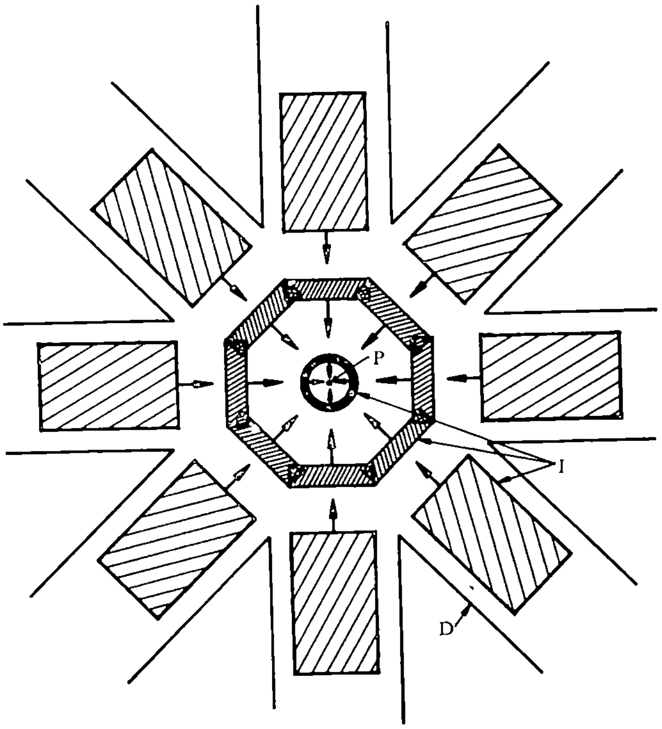

Fig 2 Coalescence and projection of several ion beams on to a thermonuclear pellet I, Ion beam, D, drift tube, P, thermonuclear pellet

the proposed ion beam technique. If the accelerating voltage is V and the ion current I then

$$v = \text{const}\ \sqrt{V} \quad (3)$$

$$\varepsilon = IV \quad (4)$$

From equations (1–4)

$$v = \text{const}\ (1 - t/t_0)^{-1} \quad (5)$$

$$V = \text{const}\ (1 - t/t_0)^{-2} \quad (6)$$

$$I = \varepsilon/V = \text{const}\ (1 - t/t_0)^{1/8} \quad (7)$$

Since the ion current is approximately proportional to the laser light intensity, a simultaneous change in both the accelerating voltage and laser light intensity can produce the power profile equation (2).

The proposed technique offers two possibilities for nuclear micro-explosions.

First, the ignition of rather large micro-explosions with two beams of ~100 MJ produced in two ~ 100 m drift tubes clashing head on, compressing and heating between them a sandwich of dense thermonuclear or fission–fusion hybrid material. For fusion pellets a minimum energy of ~ 10^2–10^3 MJ and for hybrid fission–fusion pellets somewhat less energy would be here required. It is therefore conceivable that two beams each having an energy of ~ 100 MJ would suffice.

Second, the use of many beams approximating one beam of spherical symmetry and which are projected simultaneously into a spherical combustion chamber as shown in Fig. 2. There, after having entered the chamber, the different beam pulses would coalesce into one ion pulse having the shape of a spherical shell, the thickness of which would decrease with time as the pulse approaches the centre of convergence at which the pellet to be ignited is placed. For reactor conditions a total beam energy of ~ 1 MJ would be required, as in the case of laser fusion, but since the focusing here may not be as perfect as for optical radiation a larger input energy may be needed, this does not, however, seem to present a problem since the energy can be drawn from cheap inductive storage devices. The pulse length required for solid pellet heating is of the order ~ 10^{-8} s and less. If the pellet has the form of a spherical shell the pulse can be somewhat longer. So if the pulse is delivered from the diode in about ~ 10^{-6} s it has to be compressed by a factor ~ 10^3, shortening the pulse length down to ~ 10^{-9} s. This implies a radial beam compression from an initial beam width of ~ 1 m down to ~ 1 mm. It seems that there is a fairly good chance that this can be done.

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Measurement of the speed of light

WE report here the completion of a determination of the speed of light at the National Physical Laboratory[1]. The value was obtained from the product of the measured frequency and the wavelength, determined through up-conversion, of the radiation from a CO_2 laser stabilised to the R(12) transition of CO_2 at 9.3 μm.

The frequency was determined relative to the caesium standard, using successive stages of harmonic multiplication and beat-frequency detection, with HCN and H_2O lasers used as transfer oscillators. The value obtained[2] was ν = 32,176,079,482 kHz, with a statistical uncertainty, σ_m, of 0.13 × 10^{-9}, and a systematic uncertainty of ± 0.7 × 10^{-9}. (The systematic uncertainty is less than that published previously, because only the reproducibility of the CO_2 stabilised frequency is considered, rather than the uncertainty of obtaining the unperturbed R(12) transition frequency.) The wavelength was determined using interferometric measurements on up-converted light at 0.68 μm and was related to the ^{86}Kr primary length standard, realised in accordance with the latest recommendations of the Comite Consultatif pour la Definition du Metre (CCDM). The value obtained[3] for the free space vacuum wavelength is λ = 9,317,246.348 pm, with σ_m = 1.14 × 10^{-9} and a systematic uncertainty of ± 1.4 × 10^{-9}.

The product of these results gives the value for the speed of light

$$c = 299{,}792{,}459.0 \pm 0.8\ \text{m s}^{-1}$$

The uncertainty (corresponding to ± 2.7 × 10^{-9}) is the arithmetic sum of the standard error of the mean (1.1 × 10^{-9}) and the total systematic uncertainty (± 1.6 × 10^{-9}). Each of these was derived by quadrature summation of the contributions from the frequency and wavelength measurements.

Our measurement may be compared with values derived from a frequency measurement, by Evenson *et al.*[4], of the methane stabilised He–Ne laser at 3.39 μm, and the wavelength measurements made in that and other laboratories. The available data was reviewed by the CCDM in June 1973, when the method of realising the metre through the ^{86}Kr source was reconsidered. It was decided that where asymmetry of the spectral profile is observed, the defined wavelength applies to a point midway between the peak and centre of gravity. Thus, the result of Evenson *et al.* becomes c = 299,792,457.4 ± 1.1 m s^{-1}. On consideration of wavelength measurements in four laboratories, however, the CCDM recommended[5] for general use, the value c = 299,792,458 m s^{-1}, with an uncertainty of ± 4 × 10^{-9} resulting from the uncertainties of the wavelength measurements.

The satisfactory agreement of our result with those based upon the 3.39 μm laser confirms both the recommended value for c and the reliability of frequency and wavelength measurement techniques involving infrared laser radiations.

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Accurate wavelength measurement on up-converted CO_2 laser radiation

WAVELENGTH measurements, which are part of an accurate determination of the speed of light[1], have been made on the radiation from a carbon dioxide laser, stabilised to the R(12) transition at 9.3 μm by saturated fluorescence in an external CO_2 cell, which has a measured frequency[2] reproducible to better than one part in 10^9. The problems of a direct intercomparison of infrared and visible wavelengths were avoided by mixing the CO_2 radiation with light from a 10-mW He–Ne laser at 0.63 μm to give a difference frequency sideband at a wavelength of 0.68 μm. This mixing process, known as up-conversion, was performed in a cooled, single crystal of proustite[3,4]. The wavelength of the CO_2 radiation was then determined using the relationship $\lambda_{9.3} = \lambda_{0.63}/(1-R)$, in which it is assumed that c is the same for the two visible radiations. The wavelength measurements were thus of the ratio $R = \lambda_{0.63}/\lambda_{0.68}$ and the value of $\lambda_{0.63}$ with respect to the primary length standard.

The value of $\lambda_{0.63}$ was determined by observing the frequency of beats against a He–Ne laser stabilised by saturated absorption of iodine, which is reproducible to better than one part in 10^{10}. The $^{127}I_2$ wavelength has been determined with a precision of 4 parts in 10^{10} by direct interferometric comparison with the ^{86}Kr standard[5]. Measurements from five other laboratories give a mean value within 1 part in 10^9 of our determination[6].

The wavelength ratio, R, was measured using Fabry–Perot interferometers with silvered plates and interchangeable fused silica spacers which incorporated piezoelectric adjustments. Two interferometric techniques were used, the first, and less precise, used a pressure scanning system developed from the arrangement used previously for precision measurements of wavelength[5]. For this first set of measurements the 10-mW laser was stabilised by reference to a Lamb dip laser which was ultimately referred by beats to component 'h' of the $^{127}I_2$ stabilised laser (λ = 632,991,369.6 fm). Using a spacer of 187 mm we took 37 measurements. A further 11 were taken with a 15 mm spacer. These observations lead to a value for $\lambda_{9.3}$ of 9,317,246.33 pm, with an uncertainty of ± 0.06 pm ($\pm 7 \times 10^{-9}$), which is the linear sum of random and systematic uncertainties (Table 1).

Table 1 Uncertainties of pressure scanning measurement (parts in 10^9)

Observational statistics	
	σ_m
Determination of $\lambda_{633}(^{127}I_2)$	0.4
Determination of $(1-R)$	
Short etalon	2.8
Long etalon	3.1
Resultant σ	4.2
Estimated systematic uncertainties	
Drift of Lamb dip laser (± 1 MHz)	±2
Refractive index calculations	
Pressure (± 0.1 torr)	±1
Temperature (± 20 mK)	±0.5
Nonuniformity of illumination	±1
Chromatic aberration	±0.7
Quadrature summation	±2.6

In the second, more precise, interferometric method the Fabry-Perot enclosure was evacuated. The interferometer was used with servo control of its length so as to be at a maximum of an interference fringe for the standard laser radiation, which was component 'd' (λ = 632,991,178.3 fm) of the $^{127}I_2$ stabilised laser. Using electronic feedback control to the 10-mW laser, the wavelength of the up-converted radiation was adjusted so that its interference pattern was also at the maximum of a fringe. The beat frequency between the 10-mW laser and the $^{127}I_2$ standard was counted directly. This measurement replaces the fringe interpolation necessary with the pressure scanning system. Thirty observations of the beat frequency, each of duration 10 s, were combined to give a single measurement. Using an etalon 187 mm long, 30 measurements were taken. A further 11 were taken using an etalon 19 mm long. Combined, they give a value

$$\lambda_{9.3} = 9{,}317{,}246.348 \pm 0.024 \text{ pm}.$$

The uncertainty, corresponding to $\pm 2.5 \times 10^{-9}$, is the linear sum of random and systematic uncertainties (Table 2).

Table 2 Uncertainties of servolock measurement (parts in 10^9)

Observational statistics	
	σ_m
Determination of λ_{633} ($^{127}I_2$)	0.4
Determination of $(1-R)$	
Short etalon	0.84
Long etalon	0.66
Resultant σ	1.14
Estimated systematic uncertainties	
Drift of etalon length	
Short etalon	±0.6
Long etalon	±0.3
Nonuniformity of illumination	±0.7
Chromatic aberration	±0.7
Modulation of $^{127}I_2$ intensity	±0.7
Reproducibility of $^{127}I_2$ laser	±0.2
Quadrature summation	±1.4

Differences of interferometer length, resulting from defects of flatness and parallelism, coupled with differences in the uniformity of illumination from the two sources, are important causes of error in the intercomparison of wavelengths. We measured the effect in our instrument by passing light from the 10-mW laser directly through the optical elements and diffuser normally used for the up-converted radiation. Operation of the servosystem should then result ideally in a zero beat frequency. The actual beat offset was determined using bidirectional counting, and verified as mainly systematic (7 parts in 10^9) by interchanging the optical elements with those used for the standard. The uncertainty ascribed to modulation of the standard laser results from a possible discrepancy between the time averaged frequency and time averaged wavelength when third derivative locking[5] is used.

The value determined with the servolock system is regarded as the final result of these measurements. The pressure scanning result was significantly less precise, although in excellent agreement. The wavelength standard throughout was the radiation of the $^{127}I_2$ stabilised He–Ne laser. The wavelength values assigned to it are those measured here recently[5] and in accordance with the advice of the Comité Consultatif pour la Définition du Mètre[6], relate to the 'as maintained' metre at the National Physical Laboratory. As mentioned already, that wavelength measurement, which used the ^{86}Kr source, is in excellent agreement with those of other laboratories, so that the results reported here are in direct accordance with the metre as it is internationally realised.

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A burner for mixtures of very low heat content

We must anticipate a growing demand in the future for the means to burn low grade fuels, and fuel/air mixtures which may not even be normally flammable. Upcast gases from coal seams, ventilation air from mines, exhaust gases from a wide variety of industrial processes, and lean methane/air mixtures from some fermenting wastes are examples.

When gases such as these are allowed to escape unburnt, some particularly undesirable pollutants are added to the atmosphere quite apart from the wastage of energy. The amounts are far from negligible: it has been calculated that the methane content of the ventilation air from mines would be sufficient[1] to supply alone the entire power requirements of colliery machinery in the UK.

The majority of our combustion systems, being mixing-controlled, are quite unnecessarily inefficient, polluting and archaic[2,3]. Even for premixed reactants, combustion theory has treated flame properties such as temperature in the reaction zone and thus burning velocity, flammability, and so on, in terms of the initial fuel/air ratio alone. Yet merely arranging for a variable amount of heat recirculation breaks this dependence[4]. Our present concern is the burning of very lean mixtures, and it has been shown[5] that the percentage of fuel required to render a mixture flammable, decreases almost linearly with initial temperature, for example, at −0.0045% per ° C for CH_4. A study of the rate of burning of exceedingly lean methane/air mixtures, heated by an external source, has shown[6] that preheating gases in a furnace to, for example, 1,000° C, would ensure the complete reaction of even 0.5% CH_4 in air within 10^{-3} to 10^{-2} s.

Providing this heating by the 'borrowing' of some of the product enthalpy, without simultaneous dilution of reactants by products, has been considered[4], and the beneficial effects on limiting burning rates and compositions, on pollutant emission, on fuel saving and on the efficiency of conversion to electrical power when the unconverted energy is incorporated in the heat recycle, have been calculated[7]. Although the feasibility of such a scheme was demonstrated experimentally, this was carried out using a counter current heat exchanger surrounded by a Dewar flask, all constructed in fused silica[7] which was too transparent in the infrared and somewhat removed from current practice. Calculations reveal that it is necessary to provide many surfaces between the combustion region and the ambient atmosphere, and the maximum possible heat exchanger area must be arranged so that a layer of cold reactants shields the device from its surroundings.

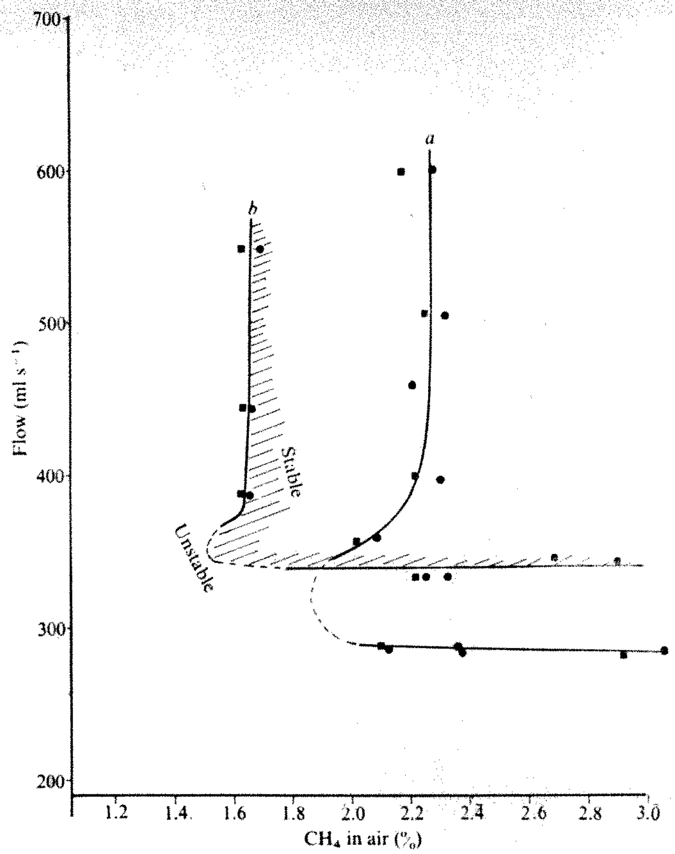

Fig. 2 Steady state stability characteristics of two burners as a function of mixture strength. Both burners are constructed of Inconel 600 strip 5 cm wide and the flow channel dimensions are 0.4 cm by 3.5 cm. *a*, 145 cm strip made into spiral of 4.5 turns; *b*, 229 cm strip made into spiral of 8 turns. ●, Stable flame; ■, unstable flame.
NOTE: The normal limit of flammability of methane is 5.3%.

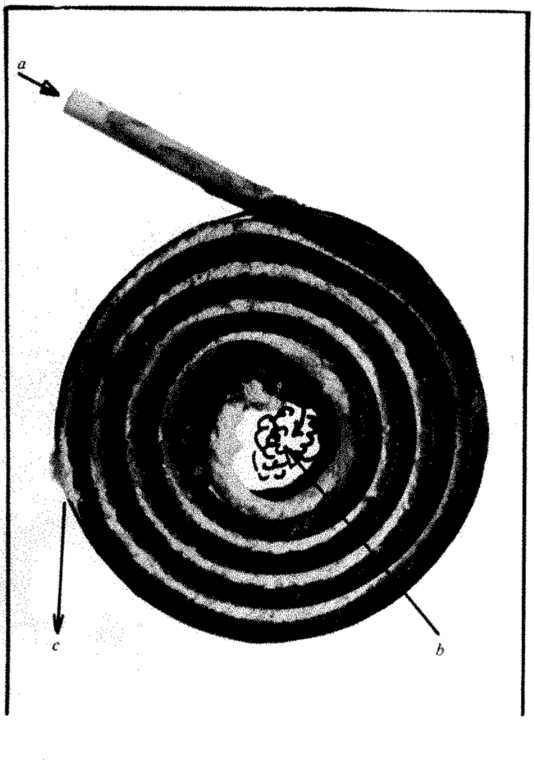

Fig. 1 Model of a burner of the type described in the text. *a*, Reactions (dark in simulation); *b*, combustion chamber; *c*, products (light in simulation).

Figure 1 shows a model of a slice of virtually the simplest device which combines these attributes. The cylindrical geometry of the burner is a compromise between the theoretical ideal of spherical symmetry on the one hand and reasonable ease of construction on the other. The experimental burners were readily constructed of strips of high temperature metal alloys (Inconel 600, Nimonic 75) by winding the spirals on spacers situated at the flat ends, which were subsequently sealed with high temperature cement. For laboratory feasibility studies, relatively thin slices (less than 5 cm open channel width) were deemed adequate. Thermocouples, igniters and any other instrumentation could be inserted before sealing instead of taking the leads out in between the spiral layers. Thermocouples at the inlet and outlet of the combustion chamber allow the position of the flame to be monitored, and indicate the amount of preheat and the heat release by combustion. A thermocouple at the exit allows the assessment of total heat losses when the exit enthalpy is compared with the chemical energy fed in. Certain ceramic materials also lend themselves to this form of construction, and would be suitable if very much higher temperatures are required.

Preliminary results were obtained with combustion chambers consisting simply of a cavity at the centre of the spiral. The reactants enter it through apertures in the cen-tial fold of the metal strip As with any other type of burner, the flame is stable for a limited range of flow rates, one difference which arises, however, is that blow-off, consequent on too high a flow, or flash-back, which occurs when the flow is too low (that is equivalent to premature ignition) decreases the effective length of the heat exchanger by moving the flame away from the centre of the spiral This introduces an automatic self stabilising mechanism against flash-back Figure 2 shows two limiting stability curves (flow velocity against fuel/air ratio) illustrating the effects of varying the length of the strip and of the geometry of the combustion chamber Even with the present small sizes (see Fig 2) extended ranges of stable burning were obtained for mixtures of fuel content corresponding to 1/3 the normal limit of flammability, and a much smaller fraction of the leanest mixtures stabilisable on conventional burners At this point the amount of heat recirculated is more than double that released by the reaction

The potentialities of such devices range from burning up undesirable pollutants, through association with fluidised beds, to direct power generation The design in which a high temperature fluidised bed combustor contained in a thoria crucible is located at the centre of the spiral, permits heat removal at the maximum temperature either by radiation or by a heat pipe Association with Stirling motors seems promising Here again the ability to vary the bed temperature independently of the fuel/air ratio and the flow velocity, by varying the amount of heat recirculated, makes it possible to maintain the most desirable conditions within the combustor Thus, poor solid fuels could be burnt at the same time as lean gas mixtures are combusted, and temperatures can be maintained below values at which concentrations of NO become appreciable

No less interesting are the possibilities regarding the direct generation of electricity In the past, magnetohydrodynamic, calorelectric, thermionic and other schemes have been considered in the field of combustion[8], but with little success Several attributes of the present burners make it seem worth while to reconsider some of these schemes One such attribute is the automatic feedback of the unconverted heat to the incoming reactants Another is the convenient geometry of the burner (for example for the application of magnetic fields) and the very large area of metal which it maintains at a high temperature with the smallest possible input of fuel In the small experimental devices under test, the ratio of the surface area of the metal strip to the cross-sectional area of the duct is over 2,000 and a throughput of about 200 W maintains approximately 0 1 m^2 of metal at white heat There can be little doubt that a thermionic generator constructed in a shape suitable for this spiral burner would yield an excellent overall efficiency[7] with lean mixtures

We carried out a simple experiment at atmospheric pressure, based on the supposition that, as the paths which the charge carriers have to cross is only of the order of 4 mm, many electrons will remain unattached[8] We coated two small electrode strips, one with barium oxide (to emit electrons) and the opposite one with potassium chromate (the positive ions from which would help to neutralise their space charge) The strips were again separated with insulating spacers, surrounded by radiation shields to simulate the coils of the spiral, and heated to various temperatures by flame gases while the currents flowing between them, and the potential, were measured The current gradually decayed with time and there was an associated deposition (presumably from the potassium chromate) on the barium oxide Potentials were of the order of 0 6–0 8 V, and graphs of log (current density) against $1/T$ gave straight lines corresponding to an activation energy of $1\ 81 \times 10^5$ J mol^{-1} Maximum current densities extrapolated to zero time and a temperature of 1,200° C were about 200 A m^{-2} These current densities are of course orders of magnitude smaller than the least values that have, in the past, been considered necessary to render viable an evacuated diode thermionic generator Our objective was not to produce a generator but merely to illustrate the feasibility of the principle, no doubt many other coatings and systems would yield better results Even so, at 1,200° C the maximum initial power density of about 150 W m^{-2} could yield an output of several tens of watts if an appreciable area of our small experimental spiral were maintained at that temperature This is to be compared with an input enthalpy flow rate of 200 W already attained in our preliminary experiments When used in this capacity, the spiral would, in any case, be built up to a large diameter so that the exit temperature, after abstraction of electrical power, need not exceed that of the incoming gas by more than the very small amount required to drive the heat transfer

We therefore conclude that devices of this kind are not only capable of burning exceedingly lean fuel/air mixtures —well below the conventional flammability limit—but may, with some experimental refinements, also lend themselves to direct power generation from such mixtures

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The stability of connected linear systems

GARDNER and Ashby[1] have given an account of the sudden onset of instability at a particular connectance in a set of N first-order linear equations, described by $\dot{x}_i = A_{ij} x_j$ They gave graphs for $N = 4$, 7 and 10, showing how the probability of stability varied with the connectivity, C, which was defined as the fraction of the coefficients A_{ij} $(i \neq j)$ allowed to be non-zero These couplings, A_{ij}, were uniformly distributed randomly between $+1\ 0$ and $-1\ 0$, and the diagonal terms, A_{ii}, were uniformly distributed between $-0\ 1$ and $-1\ 0$ This result seemed to be of great importance as a model for many real systems

As a preliminary to a study of the effects which it has been anticipated would occur if there were strongly local structure, we have repeated these calculations, but could not confirm the sharp, stepwise onset of instability Figure 1 shows our results compared with those of Gardner and Ashby[1]

Points were calculated by actually finding the eigenvalues and examining them for real parts equal to, or greater than, zero (using the Nottingham Algorithms Group subroutine for non-symmetrical real matrices) Figure 2 shows the low-connectivity region on an increased scale

R M May[3] examined the situation theoretically and concluded that the critical connectivity, C, is given, for sufficiently large N, by $a^2NC = 1$, where a^2 is the mean square value of the non-zero A_{ij} He finds that "the relative

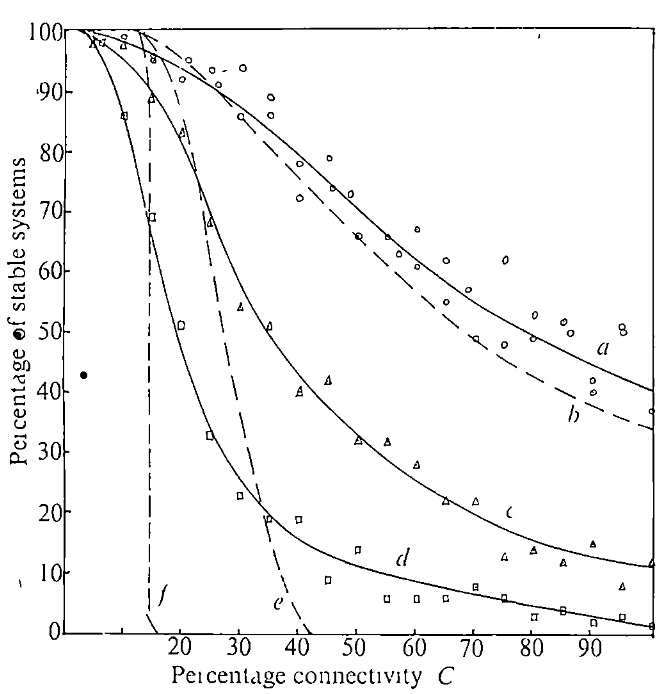

Fig. 1 Connectivity, C, against percentage of stable systems Each point corresponds to 100 randomly filled matrices, except in cases of $N=20$ Dotted lines, results of Gardner and Ashby[1], solid lines, our results a, $N=4$, b, $N=4$, c, $N=7$, d, $N=10$, e, $N=7$, f, $N=10$

width of the transition region, scales as $N^{-2/3}$"

From the slopes of the curves where they are roughly linear, we found that the width of the transition region (the inverse of the slope) is approximately proportional to $N^{-3/2}$ Ashby and Gardner[1] found a much steeper function, the width approached zero for $N = 10$

It seems that, given a number of systems which are stable in isolation from one another, increasing the connectivity (keeping equal probabilities of positive and negative couplings) simply increases the probability of instability All authors are agreed upon that general conclusion The conjecture of Gardner and Ashby, that the sudden change from stability to instability occurs at a certain critical nonzero connectivity, has not been confirmed Therefore, although earlier comments[2-4] on that particular step-like feature remain valid in their general analyses, they can be refuted once it has been shown, as it has been shown here, that the feature is an artefact of the computations of Gardner and Ashby[1]

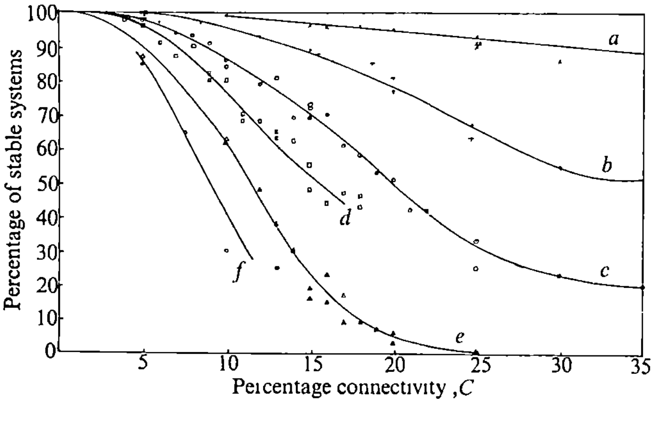

Fig. 2 Connectivity, C, against percentage of stable systems for the low-connectivity region on an increased scale Each point represents 100 repetitions except for $N=20$ a, $N=4$, b, $N=7$, c, $N=$ 10, d, $N=12$, e, $N=16$, f, $N=20$

Computer experiments on the effects of localising the connectivity are continuing

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Evolution of organic compounds in volcanic regions

According to Oparin[1], the origin of life is a natural consequence of the long evolution of organic compounds Much effort has been devoted to the abiogenic synthesis of biologically important compounds[2] but because little is known about conditions on the primitive Earth, the results of such experiments are inconclusive For this reason, the examination of volcanoes, hydrothermal systems and submarine volcanoes as sources of organic compounds seems to be highly promising

In fact, several theoretical[3,4] and experimental studies[5] have confirmed the thermal abiogenic synthesis of a wide range of organic compounds, and it may be possible to confirm the formation of organic compounds in volcanic regions

Amino acids and nucleic acid bases, such as adenine[4], can be easily synthesised in the presence of hydrogen cyanide, so the discovery of cyanide and thiocyanate compounds in areas of volcanic activity is essential if the volcanic model of the evolution of organic compounds is to be substantiated The results of examining volcanic material for the presence of some prebiological components (hydrogen cyanide and its derivatives) are given below In the search for these compounds some areas in Kamchatka and the Kuriles Islands are very suitable because intensive outbursts of juvenile material are observed in many eruptions there

From June to July 1973, the volcanic areas of the Kuriles (the Mendeleev and Golovnin volcanoes on Kunashir Island, the Alaid volcanoes on Atlasov Island) and Kamchatka (the Uzon, Malyi Semyachek and Mutnovsky volcanoes) were explored

Some thermal springs in the caldera of the Golovnin volcano (Kunashir Island) contain thiocyanates and hydrogen sulphide This indicates the primary formation of hydrogen cyanide with its subsequent transformation into thiocyanates Thiocyanates have been recorded previously[5] in other volcanic areas

In the water of several thermal springs in the caldera of the Uzon volcano (Kamchatka), cyanides were detected in the form of soluble ferrocyanides confirming some previous data[6] As might be expected, free hydrocyanic acid has not been found in these springs, because it is very reactive and because a large number of accompanying substances, such as sulphur in various stages of oxidation, are present

Free hydrocyanic acid can exist in active volcanic regions in conditions of fairly high temperatures and in the complete absence of substances that interact with it. Such conditions are found in the lava stream area on the Alaid volcano (the Kuriles) that erupted in 1972 and hydrocyanic acid was discovered in concentrations of the order of 0.01 mg l^{-1} in gas samples with a temperature of about 900° C that were taken from crevices in sheets of lava. The presence of the acid was determined by converting hydrocyanic acid to cyanogen chloride later identified using glutacondialdehyde (which forms a polymethine dye with 5,5-dimethyl-1,3-cyclohexane dionin) and also by the Berlin blue test. The presence of hydrocyanic acid derivates in volcanic exhalations has been noted before, but the detection of hydrocyanic acid proper calls for some additional discussion.

The character of the hydrogen cyanide detected is very important because it can also result, for instance, from a pyrolytic destruction of nitrogen-containing organic compounds. Nevertheless, the sampling conditions and the geological structure of the slag cone of the Olimpiiskii break (the site where gas samples were taken) in the Alaid volcano, point to the 'juvenile' character of recorded hydrocyanic acid.

The analysis of the volcanic gases and the temperatures indicate that hydrocyanic acid is a probable component of volcanic activity. The experimental data obtained can be considered as a confirmation of an earlier hypothesis about the role of volcanism (particularly submarine volcanism) and thermal springs in the processes of prebiological evolution on the primitive Earth.

Indeed, in strong eruptions, approximately 10^9 cubic metres of gas are released. If my results are correct, the amount of hydrogen cyanide liberated into the atmosphere in a single eruption would be about 10^7 g. Evidently volcanism must have played a significant role in the synthesis of organic compounds. Amino acids have also been detected in several thermal springs. But conclusions about the 'juvenile' nature of these compounds can be made only after undertaking additional investigations.

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Experimental production of diabetes mellitus by a nonendocrinal approach

THE immunofluorescent demonstration of large deposits of fibrin in the microvascular lesions[1,2], and evidence of a slow consumptive fibrinocoagulopathy in maturity onset diabetes mellitus[3,4], suggested the possibility of a primary fibrinocoagulopathic vascular aetiopathogenesis of the disorder. The present work was undertaken to investigate the possibility of an experimental production of diabetes mellitus in animals by induction of slow consumptive fibrinocoagulopathy[5].

A batch of 14 rabbits, weighing 1.5–2 kg was studied. The animals were kept on a standard pellet diet of the following composition, crude protein, minimum 20.0%, ether extract, minimum 6.0%, crude fibrin, maximum 4.0%, ash maximum 8.0%, calcium, minimum 1.5%, phosphorus, minimum 0.6%, nitrogen-free extract 48.0%, metabolisable energy, 3,000 calorie kg^{-1}. Mineral stabilised vitamin A and D_3 (Roche), vitamin E, vitamin K, vitamin B_{12}, vitamin C, thiamine, riboflavin, pantothenic acid, niacin, choline chloride, folic acid and trace minerals (cobalt, copper, iron and mangaese) were also added to this formulation. The animals were fed wih 60 g of the pellet diet and 110 g of vegetables and clean fresh water daily.

The production of 'slow consumptive fibrinocoagulopathy' was achieved by intermittent administration of intravenous bovine thrombin (Upjohn, USA) in small doses. The optimum dose of thrombin was determined by trying graduated doses of bovine thrombin from 5 National Institute of Health (NIH) units to 13 NIH units.

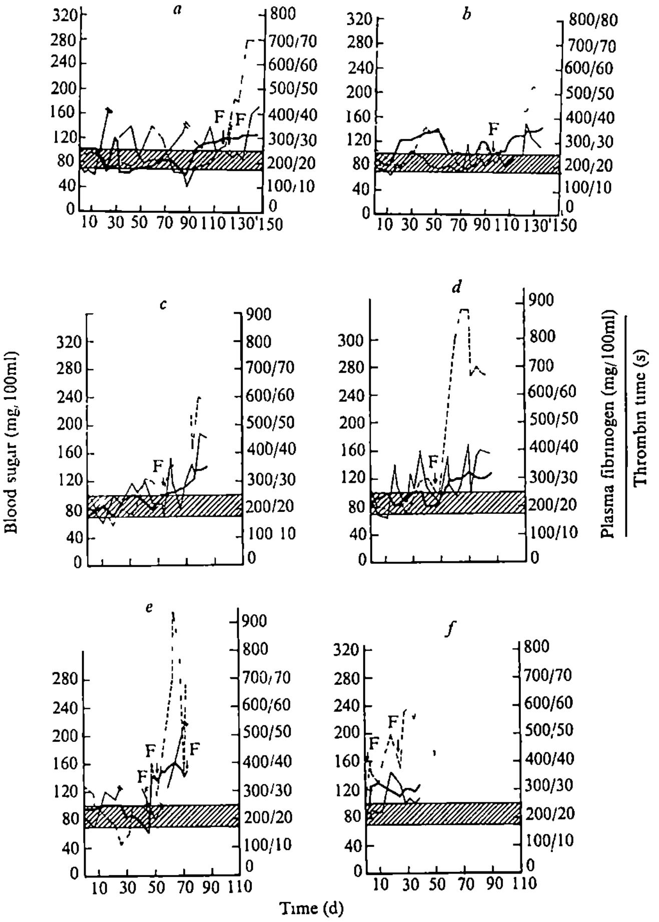

Fig 1 Blood sugar (▬▬), plasma fibrinogen (— — — —) and thrombin time (————) in six fibrinopathic diabetic animals. Shaded area shows the normal range (70–100 mg per 100 ml) of blood sugar. F, intravenous injections of bovine fibrinogen. *a*, Rabbit No 2, *b*, rabbit No 5, *c*, rabbit No 8, *d*, rabbit No 10, *e*, rabbit No 11, *f*, rabbit No 12.

While no significant alterations were observed with 5 NIH units, rapid deaths occurred with 12 and 13 NIH units. The thrombin dose was accordingly standardised at 10 NIH units, diluted in 10 ml normal saline, and administered at the rate of 1 U ml^{-1} min^{-1}. A faster rate of injection and higher concentrations of the thrombin solution produced fatal results. On this dosage schedule, it was possible to keep the animals alive in a state of fibrinocoagulopathy. The thrombin solution was routinely injected through the ear vein of the animals.

The haematological assessment for the fibrinocoagulopathic

Table 1 Results of experimental fibrinocoagulopathy in 14 animals

Rabbit No	Dose of thrombin administered	Rate of thrombin infusion	Haematological state	Clinical result
	1 Standarisation of thrombin dose and rate of infusion			
R_1	12 NIH/ 5 ml	1 ml min^{-1}	— —	Immediate death
R_3	13 NIH/10 ml	1 ml min^{-1}	— —	Immediate death
R_6	10 NIH/10 ml	Rapid injection (5 U min^{-1})	— —	Immediate death
R_9	10 NIH/10 ml	Rapid injection (2 U min^{-1})	— —	Immediate death
	2 Non fatal vascular complications			
R_7	10 NIH/10 ml	1 ml min^{-1}	Fibrinocoagulopathic state with prolonged thrombin time and reduced plasma fibrinogen (< 350 mg/100 ml)	Developed sudden hind limb paralysis after eleven injections without developing hyperglycaemia Experiment discontinued (blood sugar < 100 mg/100 ml)
	3 Experimental fibrinopathic diabetes mellitus			
R_2 R_5 R_8 R_{10} R_{11} R_{12}	10 NIH/10 ml	1 ml min^{-1}	Maintained 'Slow fibrinocoagulopathic state', with prolonged thrombin time and plasma fibrinogen above 350 mg 100 ml	Sustained diabetes mellitus (blood sugar 110–160 mg/100 ml)
	4 Central group			
R^4 R_{13} R_{14}	No thrombin injection, normal saline 10 ml injected intravenously		Normal thrombin time 20–22 min with 10 NIH units of thrombin and plasma fibrinogen 270–320 mg per 100 ml	Euglycaemic (Blood sugar 70–100 mg per 100 ml Mean 87 7 mg per 100 ml)

state was done by carrying out the thrombin time estimation by the method as described in our earlier communications[3-5], and plasma fibrinogen estimation as described by Ratnoff and Menzie[6]

After the initial induction of fibrinocoagulopathy, hypofibrinogenaemia was prevented in the animals by maintaining the plasma fibrinogen level above 350 mg per 100 ml, while on thrombin treatment, by periodic intravenous administration of 50 mg of bovine fibrinogen dissolved in 10 ml normal saline

The blood sugar estimation was carried out by the method of Asatoor and King[7] A batch of three central animals were kept on the standard diet schedule and received injections of the diluent normal saline used in the experiment A 'double blind' method with the coded blood samples, by two independant teams of laboratory workers, was adopted in carrying out the plasma estimations Results of the study of 14 animals are shown in Table 1 and in Figs 1 and 2

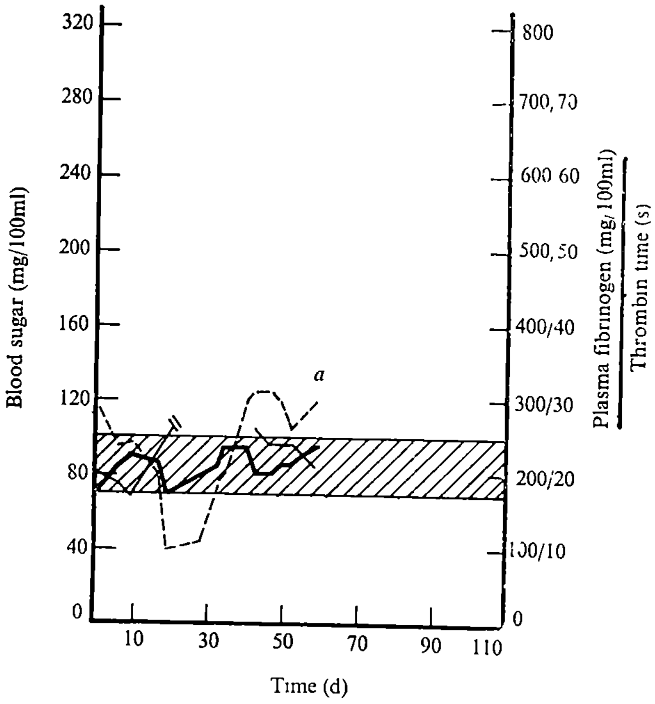

Fig 2 Blood sugar (▬), plasma fibrinogen (— — — —) and thrombin time (————) in animal R_7 Shaded area shows the normal range of blood sugar a, Acute spastic paraplegia

Our experimental procedure successfully developed a state of slow consumptive fibrinocoagulopathy in seven animals (R_2, R_5, R_7, R_8, R_{10}, R_{11}, R_{12}) and these were maintained in that state to study the alterations in the blood sugar level

In the three control animals (R_4, R_{13}, R_{14}), on scheduled diet regime, the blood sugar values ranged from 70 to 100 mg per 100 ml, with a mean value of 87 7 mg per 100 ml and the animals remained euglycaemic for the entire experimental period of 6 months, without alterations in the thrombin time or plasma fibrinogen level

In the experimental group, all seven animals showed an elevation in blood sugar values with the induction of consumptive fibrinocoagulopathy, in about 2–4 weeks' time The primary rise in the blood sugar levels, however, showed a decline with the hypocoagulability and reductions in the plasma fibrinogen concentrations In animal R_7, the primary elevation was within normal range, while the remaining six animals (R_2, R_5, R_8, R_{10}, R_{11}, R_{12}) became hyperglycaemic with values above 110 mg 100 ml

In all six animals (R_2, R_5, R_8, R_{10}, R_{11}, R_{12}) (100%), the hyperglycaemia could be reinduced by raising the plasma fibrinogen level above 350 mg per 100 ml, by supplementary injections of 50 mg of bovine fibrinogen Thereafter the animals remained diabetic, being maintained on fibrinocoagulopathic state with the plasma fibrinogen level above 350 mg per 100 ml (Fig 1)

Animal R_7, however, was not given any supplementary fibrinogen injection and the animal continued to be euglycaemic in a state of consumptive fibrinocoagulopathy till it developed acute spastic hind limb paralysis indicating a macrovascular occlusive lesion of the spinal artery (Fig 2)

In the case of R_{12}, the animal was given the supplementary dose of fibrinogen on the first day along with the primary dose of thrombin and developed a sustained diabetic state right from the very beginning, without showing any decline in the blood sugar values (Fig 1, R_{12})

The observations therefore provide convincing experimental evidence of a production of diabetic state by consumptive fibrinocoagulopathy in the animals The critical factor in developing the fibrinopathic hyperglycaemia seems to be a normal or raised plasma fibrinogen in a state of consumptive fibrinocoagulopaty in the animals As far as we are aware, this is first reported since the discovery of insulin, of an experimental production of diabetes mellitus by a nonendocrinal approach.

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Mode of action of ancrod as a defibrinating agent

ANCROD is a thrombin-like enzyme obtained from the venom of *Agkistrodron rhodostroma*. When added to plasma, ancrod initiates fibrin polymerisation by the cleavage of fibrinopeptide A from fibrinogen[1] but activates factor XIII (plasma transglutaminase) very slowly[2]. Factor XIII, when activated, catalyses the formation of crosslinks between the α chains and between the γ chains of fibrin[3]. The absence of these crosslinked chains, especially the α chain polymers, allows fibrin to be easily degraded by plasmin[4,5]. Thus it is believed that ancrod acts as a defibrinating agent *in vivo* by producing non-crosslinked easily lysable fibrin deposits. We wish to present some preliminary evidence to show that commercial preparations of ancrod activate factor XIII in plasma *in vitro* and may also mediate the conversion of prothrombin to thrombin. In addition a hypothesis is presented to explain the defibrinating effect of ancrod *in vivo*.

Human fibrinogen (Grade L from KABI, Stockholm, Sweden) and bovine (Diagnostic Reagents, Thame, Bucks) or human (Parke-Davis) thrombin were used in these experiments. Human plasma was collected in 0.38% sodium citrate and stored in aliquots of 1 ml at −70° C when not used immediately. Hirudine, an inhibitor of thrombin activity, was obtained from Sigma, Surrey, and a naturally prothrombin deficient plasma was donated by Dr P. Barkhan (Guy's Hospital, London) through the offices of Dr M. Brozovic of this Institute. This prothrombin deficient plasma was immunologically and biologically devoid of prothrombin activity. Clinical grade ancrod (Arvin, batch F 1623) and a more highly purified extract (IRC 50 Arvin, batch DRP 1/143) were donated by Mr G. W. Tunnah of Twyford Laboratories, Beckenham, Kent.

Sodium dodecyl sulphate polyacrylamide (SDS–PA) gel electrophoresis was performed by the method of Weber and Osborn[6]. This technique has been successfully used to examine the subunit composition of the degradation products of fibrinogen by plasmin[7] and the degree of crosslinking of the γ chains and α chains of clotted fibrin[3]. Artificially made ($BaSO_4$ absorption) prothrombin-deficient plasma was treated separately with ancrod (40 Twyford units per ml plasma, 9 mM $CaCl_2$) and thrombin (2 NIH units per ml plasma, 9 mM $CaCl_2$) as was a 0.3% prothrombin-free fibrinogen solution (0.9% NaCl–0.4% Na citrate). After incubation at 37° C for 2 h the plasma fibrins were washed in saline–0.2 M ε-aminocaproic acid (ε–ACA) and reduced overnight in 4 M urea–1% SDS–0.2 M β-mercaptoethanol before examination by electrophoresis. The results are shown in Fig. 1. The γ chains are crosslinked and there are no α chain polymers in the Arvin-mediated plasma fibrin; however an α chain fragment of molecular weight 38,000 is evident (Fig. 1*a*)[1,8,9]. The IRC–50 Arvin-treated plasma fibrin was made up of β chains, γ dimers, some α polymers and some α chain fragments (molecular

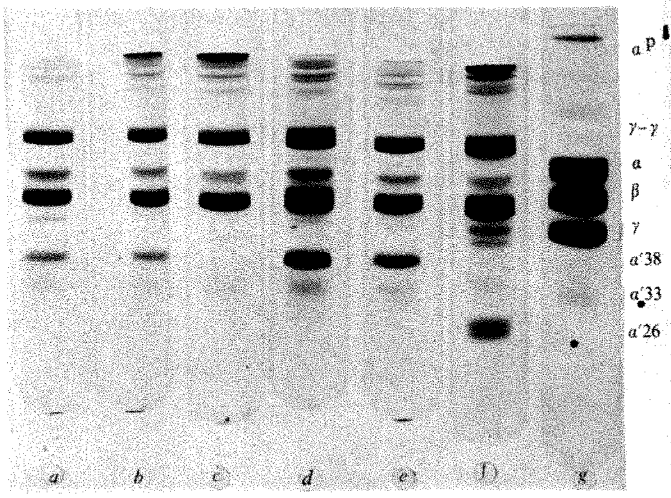

Fig. 1 Sodium dodecyl sulphate polyacrylamide (SDS–PA) electrophoresis (7% gel) of polypeptide chain subunits of fibrins obtained from plasma and from purified fibrinogen by treatment with ancrod preparations and thrombin. *a*, Plasma fibrin obtained by Arvin, batch F1623; *b*, plasma fibrin by IRC–50, batch DRP 1/143; *c*, plasma fibrin by thrombin; fibrins from purified fibrinogen by *d*, Arvin, batch F1623; *e*, IRC–50 Arvin, batch DRP 1/143; *f*, thrombin. Pattern *g*, show the three chains of fibrin α, β, γ. Electrophoresis was conducted for 16 h at 2 mA per gel. The gels were stained for 3 h with 0.25% Commassie blue in methanol/aceticacid/water (45/5/50) and destained in the same solvent. * Molecular weight ×10⁻³.

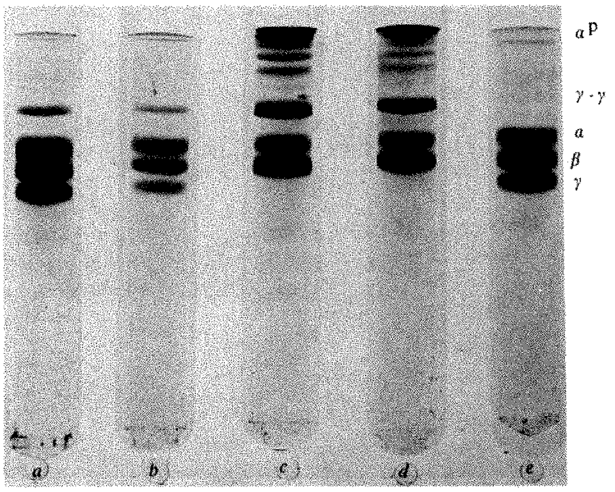

Fig. 2 Polypeptide subunits on SDS-PA gel (7%) electrophoresis of plasma fibrin obtained by IRC-50 Arvin from *a*, plasma containing no prothrombin; *b*, normal plasma with hirudine; *c*, normal plasma. Pattern *d* shows chains of fibrin obtained from plasma with thrombin; and pattern *e* shows the chains of non-crosslinked fibrin. Other details as in Fig. 1 legend.

weight 38,000) (Fig. 1*b*). The thrombin–fibrin from plasma contained β chains, γ chain dimers and α chain polymers but no α chain fragments (molecular weight 38,000) (Fig. 1*c*). Figure 1 (*d-f*) shows that high levels of Arvin, IRC–50 Arvin and thrombin cause activation of factor XIII to crosslink most of the γ chains of purified fibrin; also that both ancrod preparations lyse the α chains of fibrin to form a 38,000 molecular weight fragment while thrombin does not. Thrombin produces some α chain fragments of molecular weight about 26,000 which have been described elsewhere[10].

Plasma naturally deficient in prothrombin was treated with a clinically used level of IRC–50 (0.07 Twyford units ml^{-1}) in the presence of 9 mM $CaCl_2$. Normal plasma, containing 250 units of Hirudine ml^{-1}, was treated with IRC–50 Arvin while normal plasma samples (1 ml) treated separately with 0.07 Twyford units of IRC–50 Arvin and 0.25 NIH units of thrombin, were used as controls. The fibrins were examined for their subunits on SDS–PA electrophoresis and the results are shown in Fig. 2. It is evident that Arvin IRC–50 by itself slowly activates factor XIII (as shown by the presence of some γ chain dimers) when there is no prothrombin present (Fig. 2*a*) or when thrombin is totally inhibited by hirudine (Fig. 2*b*). It is notable that at these concentrations of ancrod no α′ chain fragments (molecular weight 38,000) are produced and no α chain polymers are found during the 2 h incubation when the prothrombin–thrombin complex is absent or inhibited. In normal plasma (prothrombin present) the subunit pattern (Fig. 2*c*) with low level Arvin is the same as that obtained with thrombin (Fig. 2*d*). It can be seen that again there is no α chain fragment (α′ 38) formed while α polymerisation (α^p) is nearly complete. This suggests that the enhanced crosslinking induced by Arvin in normal plasma is a result of thrombin activation and that low levels of Arvin activate factor XIII slowly and do not lyse fibrin α chains. The α′ 38 fragment reported here (Fig. 1) and elsewhere[2,8,9] is an effect *in vitro* of high levels of ancrod and thus could play no part in the susceptibility of Arvin-fibrin to lysis by plasmin *in vivo* as has been suggested[8]. It is emphasised that calcium levels (< 9 mM) in these experiments were such as not to clot normal plasma during the course of these experiments.

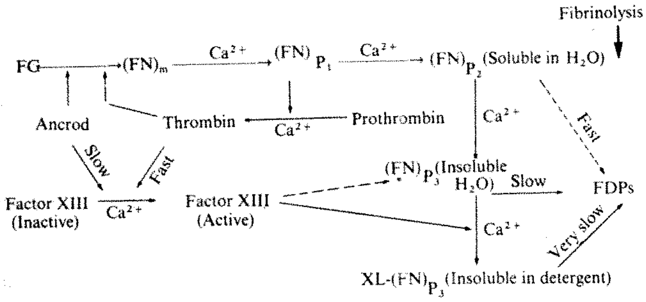

Fig. 3 Hypothetical scheme of ancrod generation of fibrin (FN) from fibrinogen (FG) *in vivo* and the ability of the fibrinolytic system to lyse the various possible fibrin polymers ($P_1 < P_2 < P_3$) to fibrin degradation products (FDPs). XL = crosslinked α chains and γ chains.

It is evident that low levels of the above ancrod preparations generate fibrin which is more rapidly cross linked in normal plasma than in plasma which does not contain prothrombin (Fig. 2). We suggest that by a mechanism as yet unknown these ancrod preparations may generate thrombin assuming that, in plasma, only thrombin can activate factor XIII. The proteolytic attack by ancrod on the α chains of fibrin (Fig. 1) does not occur at a low ancrod concentration (Fig. 2) and it can be shown that the α chains can crosslink slowly to α polymers. We have recently described[5], a fragment called D-dimer, which is formed during the lysis of fibrin clots and which contains the γ-γ crosslinks of the originating fibrin. This fragment has been also shown[11] to be released from crosslinked clots of whole plasma. But plasma from patients undergoing defibrination treatment with Arvin does not contain any of this D-dimer fragment (our unpublished results). The idea, based on *in vitro* evidence, that some soluble fibrin monomer complexes can be crosslinked by their γ chains[12,13] was not supported as we could not detect D-dimer in the plasmas of patients undergoing Arvin treatment. Since the deposition of fibrin and the formation of some γ-γ crosslinks take place at about the same time it seems that Arvin does not cause the deposition of fibrin *in vivo*. Instead fibrin, while still soluble, seems to be lysed by the fibrinolytic system in plasma.

The sequence of reactions outlined in Fig. 3 presents our current views on ancrod as a defibrinating agent, and other features of 'normal' fibrinolysis. Many aspects of this scheme need to be tested *in vitro* and *in vivo*. The main features of this hypothetical scheme are that fibrinolysis can most readily degrade soluble (under physiological conditions in plasma) fibrin polymers of size P_2 to fibrin degradation products (FDPs), that the 'lysability' of fibrin of an 'insoluble size' (P_3) and containing some crosslinks is decreased, and that totally crosslinked fibrin, XL(FN) P_3, is resistant to plasmin degradation to FDPs. It is proposed that an intermediate size fibrin polymer, $(FN)_{P_1}$ might be responsible for the activation of plasma prothrombin noted in the presence of ancrod preparations (Fig. 2). The suggestion that fibrin activates prothrombin has been expressed elsewhere[14], while both crude Malayan pit viper venom and a purified component of the latter can activate factor X[15,16]. The newly generated thrombin would then be available to augment the weak factor XIII activation potential of ancrod and would also explain why *in vivo* low levels of ancrod can defibrinate plasma so rapidly. The scheme also suggests that when normal fibrinolysis is depressed there is a greater probability of *in vivo* formation of fibrin deposits which may be crosslinked. Recent work[17] using the rabbit experimental model partly supports this aspect of the hypothesis. On the other hand it should be noted that Arvin, during infusion into man, has not caused[18] the deleterious side effects which might be expected from the *in vivo* generation of thrombin.

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Differential effects of electrical stimulation on release of ^{3}H-noradrenaline and ^{14}C-α-aminoisobutyrate from brain slices

AMINO acids, especially γ-aminobutyric acid (GABA), glutamate, aspartate, glycine and taurine have been postulated to act as central neurotransmitters in vertebrates[1,2] Aside from GABA, which seems to have met the stringent criteria necessary for establishing its neurotransmitter role[3-6], the evidence for their role is mainly circumstantial Part of it is based on electrically induced release of amino acids from slices[7], chopped tissue[6,8,9] and synaptosomes[10] derived from different portions of the central nervous system In several of such studies, there are released not only the amino acids active as neuronal excitors (glutamate and aspartate) or inhibitors (glycine and taurine), but also amino acids with no known effects on the electrical activity of neurones such as lysine, arginine, leucine, cycloleucine, proline and glutamine[7,8] There is no criterion for believing that these last are transmitter susbtances

We have studied the conditions under which electrical stimulation is a valid method for releasing neurotransmitters from brain slices For this we have chosen to study simultaneously the efflux from neocortical thin slices of a known transmitter, ^{3}H-L-noradrenaline (NA) and a known amino acid non transmitter, ^{14}Cα-aminoisobutyrate (AIB) We repeatedly stimulated the slices electrically, with increasing intensities This we call the 'voltage profile' approach Both NA and AIB are actively transported into the cells by sodium-dependent processes NA by a specific transport system into noradrenergic cells[11], when its concentration is low (under 10^{-6} M), and AIB into both neurones and glia[12] through a transport system that shows preferential affinity for small neutral amino acids[13]

The release of ^{3}H-L-NA from unstimulated slices is similar to that reported previously for ^{3}H-DL-NA (ref 14) Two different first-order components may be distinguished A fast initial one represents the washout of extracellular NA, plus NA metabolities The second component is much slower and is believed to represent the efflux of NA from intracellular stores The efflux of ^{14}C-AIB follows a similar pattern, although the initial component is much less marked than that of NA, probably because of the nonmetabolisable nature of this amino acid

After the first efflux component became negligible (that is, after 40 min) the slices were stimulated with increasing voltages (Fig 1*a*) At low voltages (0 5–3 V), only ^{3}H-NA was released, in an amount roughly proportional to stimulus strength, at higher voltages the specificity of the response was lost, and both NA and AIB were released by electrical stimuli Under

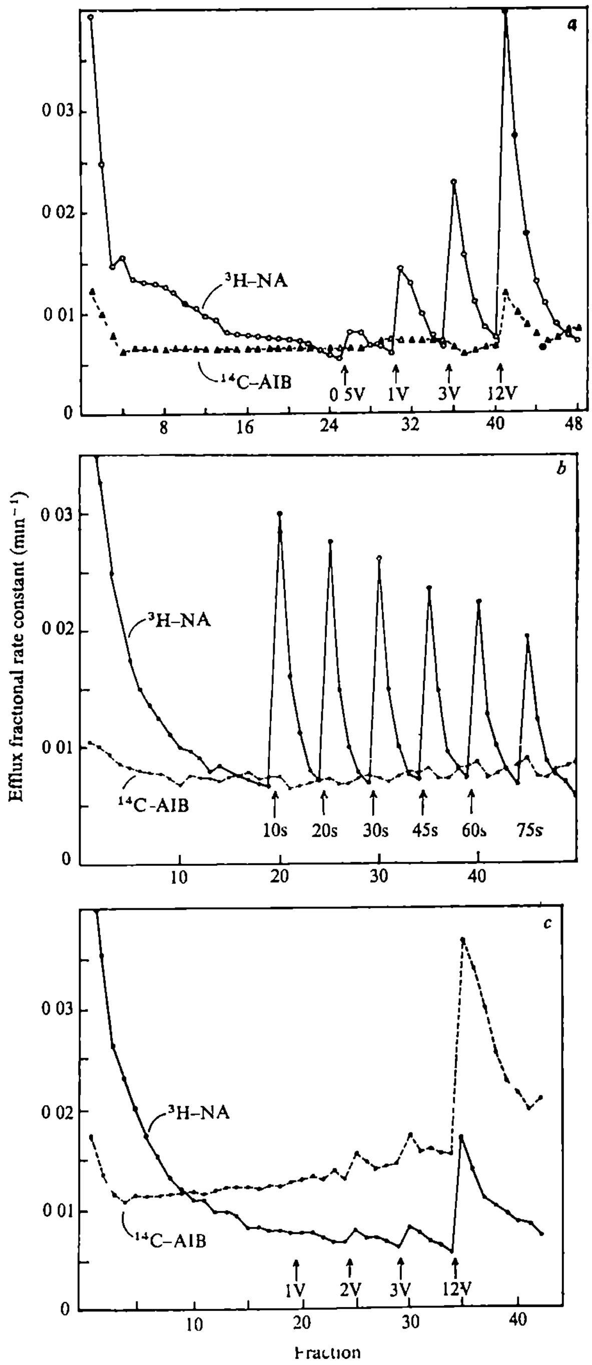

Fig 1*a* Electrically induced release of NA and AIB Slices were obtained from 120 to 150 g Sprague-Dawley rats, with blade and blade guide[15,16] Only the outermost slices were used Slices of both hemispheres remained attached and could be floated together The slices were initially incubated for 60 min in a Dubnoff shaker (37° C, 100 cycles min^{-1}) in 25 ml Erlenmeyer flasks containing 1 ml of a physiological glucose-salt solution[17], equilibrated with 5% CO_2 in O_2, and 7 8 × 10^{-7} M ^{3}H-L-NA (5 μCi, specific activity 6 4 Ci mmol^{-1}) and 10^{-4} M ^{14}C-AIB (1 μCi, specific activity 10 mCi mmol^{-1}) After this period, in which both NA and AIB were actively transported into the cells, the slices were floated into a McIlwain quick transfer electrode, and this was placed in a 30 ml beaker containing 4 ml of the same salt solution, but without ^{3}H-NA or ^{14}C-AIB The beaker was kept at 37° C in a thermoregulated water bath and vigorously gassed with 5% CO_2 in O_2 Every 2 min the fluid was aspirated with a 2 5 mm polyethylene tube connected at one end to a 3 mm Pyrex tube soldered to the bottom of the beaker, and at the other end to one of two perforations of a neoprene stopper fitted tightly to a 16 × 160 mm test tube The other perforation in the stopper connected the tube, through Tygon tubing and three-way valve, to a water pump After each aspiration the stopper was changed to a contiguous empty test tube The beaker fluid was replenished by means of an automatic syringe that aspirated the fluid from a reservoir and injected it, through latex tubing connections, to one of the two openings of a neoprene stopper placed on another 16 × 160 mm test tube, immersed in the water bath From this test tube, used for thermal equilibration, the fluid passed through a latex tube connected to a 3 × 80 mm Pyrex tube that was placed in the beaker The slice was stimulated with sine wave a c current, 50 cycles s^{-1}, obtained from the mains line and passed through two Variac variable transformers placed in series The stimulation voltage was monitored with a Tektronix model 532 oscilloscope, with its horizontal sweeping suppressed The voltage then appeared as a vertical line on the screen whose height represents twice the magnitude of the maximum instantaneous voltage (MIV) All voltages are expressed as MIV At the end of the experiment the slice was homogenised in 4 ml incubation fluid One millilitre of each collected fraction and 0 1 to 0 2 ml of the slice homogenate (with the total volume made 1 ml with incubation fluid), were counted with 10 ml of a mixture of toluene, containing 4% PPO and 0 05% POPOP, and Triton X-100 (2 1 v/v), in a two-channel liquid scintillation spectrometer Background substraction, and the calculation of ^{3}H and ^{14}C counts in each channel were performed with an IBM digital computer The efflux of ^{3}H-NA and ^{13}-C-AIB were finally expressed as efflux fractional rate constants[9] *b*, Slice stimulation with constant voltage and increasing times *c*, Stimulation in medium with no added calcium The initial incubation was done in normal glucose-salt solution, while the superfusion was in medium nominally without calcium In a second experiment, identical results were found, except that no NA or AIB were released with stimuli of 0 5 V, 30 s, or 2 V, 10 s

our experimental conditions, the limits between which electrical stimulation was a valid tool for neurotransmitter release was between 0 5 and 3 V (Fig 2) At and above 4 V, AIB, and probably all actively transported nontransmitter amino acids, are released

When the slices were stimulated repeatedly with 3 V for different lengths of time (Fig 1*b*), a progressive decline in the induced ^{3}H-NA efflux, quite analogous to synaptic fatigue became apparent With stimulation times longer than 30 s, small increases in AIB efflux could also be elicited In stimulated slices, the efflux of AIB assumed an undulating character, not seen in the absence of stimuli, that might be related to fluctuations in the concentrations of intracellular sodium

Markedly different results were obtained when the efflux of NA and AIB was studied in slices superfused with calcium-free medium (Fig 1*c*) The efflux of ^{3}H-NA did not increase when the slices were stimulated with 0 5 and 1 V, and the extra efflux induced by higher potentials was greatly reduced ^{14}C-AIB was however now released by stimuli as weak as 2 V, and at 12 V, its stimulated efflux is considerably larger than when calcium was present Independently of the stimulation voltage, the efflux of AIB also now showed a steady increase with time

The most straightforward interpretation of the electrically induced release of AIB is based on the sodium hypothesis[18] of active amino acid transport Release seen at high stimulation potentials is probably due to increased intracellular sodium[19] that enhances the affinity for AIB of the transport carrier on the inside of the membrane Similarly, it is known that in calcium-deficient media, sodium influx during stimulation is increased [20] This should also lead to an enhanced amino acid efflux during stimulation Finally, independently of stimulation, brain slices placed in media nominally without calcium[21] steadily increase their intracellular sodium[17] This correlates well with the steady increase in AIB efflux seen in calcium-free medium

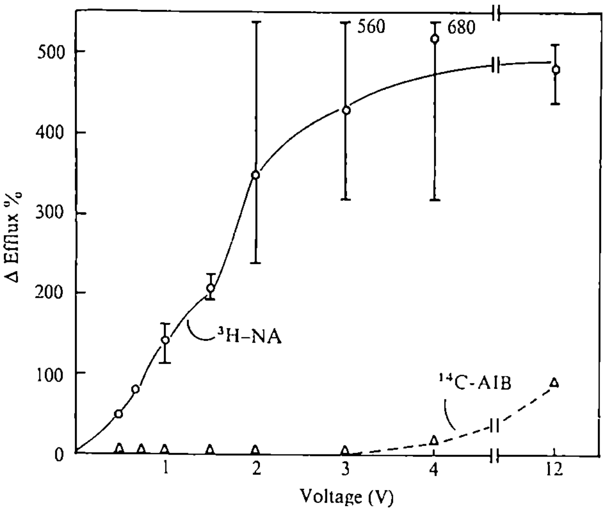

Fig 2 Differential effects of electrical stimulation on NA and AIB release Each point represents 3–5 estimations Ranges are shown as vertical lines All the triangles in the 0 to 3 V region have zero values

It seems likely that the electrically induced release of NA seen at low stimulating potentials is not immediately dependent on the reverse operation of a sodium activated, carrier mediated, transport system, but represents true calcium-dependent exocytotic neurotransmitter secretion With more intense stimuli, both calcium-dependent and sodium-dependent mechanisms may be set in operation The small electrically induced NA release seen in medium that lacks calcium could be interpreted as being due to the presence of residual amounts of calcium which still support some secretion But the strict parallel that exists under these conditions between the electrically induced release of NA and of AIB, strongly suggests that all of the NA released in the absence of calcium is due to the reverse operation of the sodium-activated NA membrane transport system The source of NA is presumably free NA present in the cytoplasm The coexistence of sodium-dependent and calcium-dependent neurotransmitter release processes, in these *in vitro* preparations, with a great enhancement of the sodium processes when no calcium is present, makes it very difficult to demonstrate an absolute calcium requirement for neurotransmitter release For this, stimulation conditions must be very carefully controlled, and there should be internal controls for sodium-dependent processes, as is the case with AIB efflux

The voltage profile approach is essentially a qualitative procedure, by which substances released by electrical stimulation may be classified as neurotransmitter-like or amino acid-like, the actual amount of substance released is, in itself, irrelevant for this classification This approach may be applied to any of the various systems used for superfusing and stimulating nerve tissue preparations *in vitro*, although the stimulation parameters will vary considerably from one system to another Its use will determine whether many of the substances reported to be released by electrical stimulation behave in a transmitter-like or in an amino acid-like manner This approach may however only be used with stimuli of alternating polarity, because monophasic pulses rapidly polarise the electrodes

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Insulin unmasks latent sodium pump sites in frog muscle

INSULIN stimulates the movement across the cell membrane of a variety of substances such as glucose[1], amino acids[2], and alkali cations Na^+ and K^+ (refs 3 and 4) All these substances move through specific membrane sites but it is not known whether insulin stimulates transport by increasing the available number of sites or by increasing the efficiency with which the sites operate

The alkali cation transport system, the Na-K ATPase, is well suited to explore this problem The Na-K ATPase is selectively inhibited by ouabain[5,6] By the use of ^{3}H-ouabain it is possible to determine the extent of the binding of the inhibitor and estimate the number of transport sites, while the alkali cation transport can be assayed by measuring the transport of labelled cations

We have studied the effects of insulin on ^{3}H-ouabain binding and ^{22}Na efflux of frog muscle Paired sartorii dissected from the same frog (*Rana pipiens*) were used to provide control and test muscles The techniques used to measure the efflux of labelled sodium have been described several times[7,8] An apparatus that transfers the muscles automatically from container to container was used[9] ^{3}H-ouabain (New England Nuclear) binding was measured in the following manner after equilibrating the muscles in the labelled solution for 50 min, they were transferred through six containers, 10 min in each, containing ouabain-free Ringer solution at 0° C Insulin-treated muscles were incubated in Ringer with insulin for 1 h before being exposed to a solution containing both insulin and ^{3}H-ouabain Excess fluid was removed and the muscles were weighed in a Mettler H10 balance Then they were digested with NCS and counted in a toluene mixture on a Beckman LS150 liquid scintillation counter Quenching was determined by the External Standard-Channels Ratio method In all experiments the concentration of insulin used was 250 mU ml^{-1}

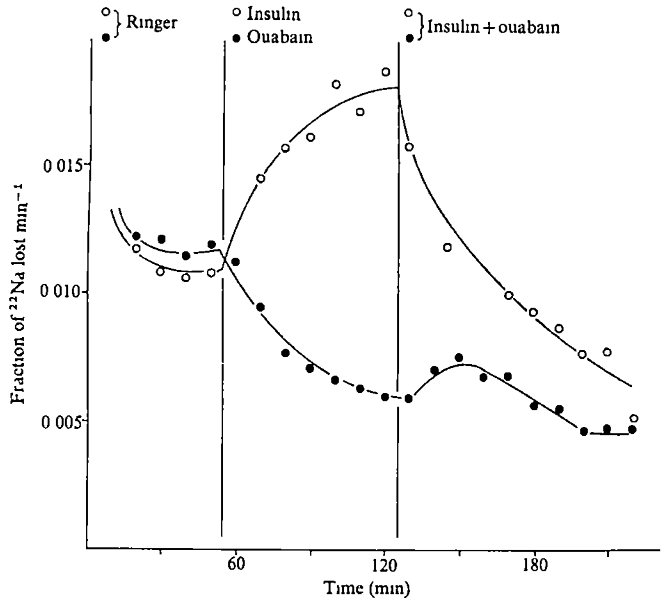

Fig 1 The effects of ouabain on the stimulation of sodium efflux caused by insulin After a control period the control muscle (●) received ouabain (1 × 10^{-6} M) and the test muscle (○) insulin (250 mU ml^{-1}) Then both muscles were transferred to solutions containing both ouabain and insulin

Figure 1 shows the stimulation of sodium efflux caused by insulin and its inhibition by ouabain The inhibitor reduced sodium efflux in the control muscle to a level equal to 0 50 times the resting value This is in agreement with previous observations which show that only about half of the Na efflux in frog muscle is ouabain sensitive, the remaining efflux consists mostly of an exchange diffusion process[7,10,11] The subsequent addition of insulin resulted only in a small and transient increase in sodium efflux In the test muscle insulin increased sodium efflux to 1 86±0 10 times (n = 5) the control level After the addition of ouabain, the efflux dropped to a level similar to that observed in the control muscle

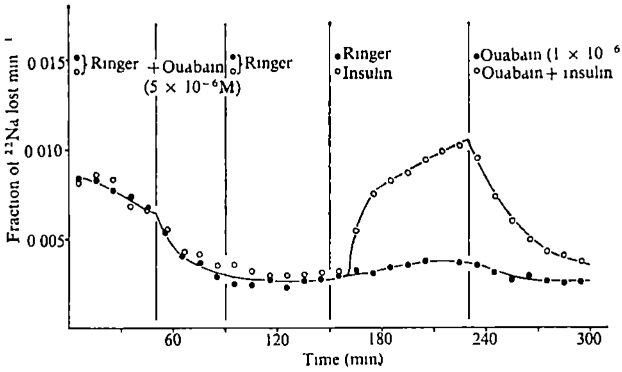

Fig 2 First both muscles were immersed in 5×10^{-6} M ouabain solution, then washed in normal Ringer for 60 min The test muscle (○) was then transferred for 80 min into a solution containing insulin and finally ouabain 1×10^{-6} M was added to the insulin containing solutions The washout of the control muscle (●) was continued in control Ringer until 140 min were completed, then ouabain (1×10^{-6} M) was added

The difference in ouabain binding between paired control muscles was always less than 5% In the experiments on the effects of insulin on ^{3}H-ouabain binding, we used two concentrations of ouabain, 2 5×10^{-7} M and 1×10^{-6} M, which inhibit 40% and 100% of the ouabain sensitive efflux, respectively

Table 1 shows that insulin produced a large increase in ouabain binding The ratios of ouabain binding by insulin treated over control muscles averaged 1 7 The average difference in binding between control and insulin treated muscles was significant for both concentrations of ouabain The scatter in the binding figures arises because there are substantial differences in the amount of ouabain bound by muscles obtained from different frogs

The stimulation of both sodium efflux and ouabain binding strongly suggests that insulin may increase the number of active sodium pumping sites in the muscle membrane To test this possibility further, we performed some experiments based on the observation that the inhibition of the Na-K pump of frog muscle caused by ouabain is very slowly reversible[12,13] Thus one can block all the pumping sites in a resting muscle, then transfer it to an ouabain-free solution and determine whether insulin will still increase sodium pumping Figure 2 shows one out of five experiments performed following this scheme After a control period, the muscles were transferred to solutions containing ouabain (5×10^{-6} M) This concentration abolished the ouabain-sensitive component of sodium efflux The muscles were then washed for 60 min in ouabain-free Ringer solution This treatment does not remove ouabain from specific sites, since there is no recovery of the pump The small increase in sodium efflux observed in Fig 2 after washing the control muscle for 140 min in ouabain-free Ringer is the largest recovery we have observed in these and another series of experiments[12] In the five control muscles of the present series, the ratio of the efflux value measured after washing in ouabain-free Ringer for 140 min over the lowest value measured during the action of ouabain was 1 06±0 05 When insulin was added to the muscle a large increase of sodium

Table 1 Effects of insulin on ^{3}H-ouabain binding

Ouabain concentration	2.5×10^{-7} M	1×10^{-6} M	'Preincubated' 1×10^{-6} M
Control	1661 ± 304	4037 ± 554	1153 ± 325
Insulin	2735 ± 499	6639 ± 825	2700 ± 336
Δ (I-C)	1074 ± 218 $P < 0.01$	2602 ± 683 $P < 0.02$	1546 ± 91 $P < 0.001$
I/C	1.66 ± 0.08	1.71 ± 0.21	3.03 ± 0.66

All binding figures are expressed in d.p.m. per mg/wet weight. Values are averages ± s.e. of the mean ($n = 5$). The specific activity of ouabain was 11.7 Ci mmol^{-1}. Δ(I-C), The difference in binding between insulin-treated and control muscles, P values were calculated using Student's paired t test. I/C, The ratio of the binding of the insulin treated over the control muscles.

efflux was observed. The increase was equal to 0.76±0.10 times ($n = 5$) the resting level. This stimulation of sodium efflux was inhibited when ouabain (1×10^{-6} M) was added to the washout solutions.

Another group of experiments of the same design as those illustrated in Fig. 2 was carried out to measure ouabain binding after saturating the sites available in resting muscle with unlabelled ouabain. They are summarised in Table 1 (preincubated). After immersing both the control muscles and test muscles in 5×10^{-6} M unlabelled ouabain (40 min) they were washed in inhibitor-free Ringer. The test muscles were then equilibrated with insulin and during the final period 1×10^{-6} M ^{3}H-ouabain was added to the solutions. The control muscle also received ^{3}H-ouabain in the final period.

Table 1 shows that preincubation with unlabelled ouabain reduced ^{3}H-ouabain binding in control muscles to about 30% of the value observed in muscles that were exposed directly to ^{3}H-ouabain. The binding remaining after the muscles had received enough ouabain to block all the Na active transport, probably represents binding to sites not linked to the cation transport process, that is unspecific binding. Indeed our own measurements of ^{3}H-ouabain binding by control muscles (to be published in detail elsewhere) show that in muscle cells, as in other cell types[14], ^{3}H-ouabain binding has two components, one associated with inhibition of Na pumping sites and another which represents nonspecific binding. Table 1 also shows that the addition of insulin to muscles preincubated with unlabelled ouabain resulted again in a large and very highly significant ($P < 0.001$) increase in ^{3}H-ouabain binding. The last two columns of Table 1 show that the increase in ^{3}H-ouabain binding caused by insulin was smaller in muscles preincubated in unlabelled ouabain than in muscles that were not preincubated with the unlabelled inhibitor. In principle one would expect that the increase in labelling after insulin treatment should be the same in both experimental groups. We believe that the quantitative disagreement with this expectation is due in great part to the scatter in ouabain binding between muscles obtained from different frogs mentioned previously, since the difference in insulin-induced binding between both groups is not statistically significant ($P > 0.1$).

In any case, the parallel augmentation in ouabain-sensitive Na efflux and ouabain binding in muscles whose resting Na pump sites had been blocked by preincubation in unlabelled ouabain suggests that both phenomena are linked and provide further support for the notion that insulin acts by increasing the number of sodium pumps in the muscle membrane.

It could still be argued that insulin destabilises the ouabain-enzyme complex. The dissociation of the complex would result in the termination of the inhibitory effects of ouabain on the sodium efflux and would make available for binding those sites previously occupied by unlabelled inhibitor. This possibility is ruled out by two observations. First, that ^{3}H-ouabain binding measured without previous exposure to unlabelled ouabain is stimulated by insulin and second, that after equilibrating four pairs of muscles with ^{3}H-ouabain for 50 min, the test muscles were washed for 60 min in solutions containing insulin while the paired control muscles were washed in Ringer solution. Washing with insulin did not decrease the amount of bound ^{3}H-ouabain. The ratio of ^{3}H-ouabain remaining in the insulin-washed muscle over the Ringer-washed muscle was 1.008±0.04 ($n = 4$).

Assuming that one ouabain molecule blocks one pump site, that inhibition of the pump is directly proportional to the number of ouabain molecules bound per cell and a surface weight ratio of 0.415 cm^2 mg^{-1} in frog muscle[8], we arrived at a number of 1,500 pumping sites per square micrometre of membrane of control muscles for both concentrations of ouabain shown in Table 1. The estimates of membrane area consider only the cylindrical surface area of the muscle, since it is possible that pump sites are also present in the transverse tubular system, the number of pump sites per unit area could be overestimated by a constant factor. The calculation involves also the assumptions that 90% of the binding was specific when 2.5×10^{-7} M ouabain was used and 70% was specific for 1×10^{-6} M ouabain. These figures were estimated from our measurements of the kinetics of ouabain binding by control muscles and following criteria developed from observations in HeLa cells[11], since binding of ouabain shares many common features in both types of cells.

The most interesting conclusion of these experiments is that insulin stimulates sodium pumping by increasing the number of pumping sites in frog muscle. This increase in number of pumping sites could result either from unmasking of latent pump sites or from synthesis of new enzyme. We found that the increase in ouabain binding caused by insulin was unaffected by the protein synthesis inhibitor cycloheximide (100 µg ml^{-1}). This observation suggests that the latter possibility is not likely.

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Surface and cell membrane activities of leukocyte chemotactic factors

PROTEINS with an affinity for air–water interfaces may also show an affinity for hydrophobic sites in cell membranes. In exploring the basis for recognition in leukocyte chemotaxis, I and my colleagues have previously reported that proteins which excite a locomotor response in leukocytes do so by virtue of their possession of externally positioned nonpolar groups[1-3]. A protein may possess such groups either because it has become denatured, because nonpolar groups have been conjugated to it, or because, like α_s- or β-caseins, its intrinsic content of nonpolar residues is too high for them all to be packed internally[4]. These proteins would be expected to be surface active and could possibly penetrate the phospholipid bilayer of a leukocyte to activate the cell for chemotaxis in a way that a more hydrophilic protein could not. This paper presents evidence that the surface activity and the chemotactic activity of proteins are indeed related, and that the chemotactic response of the leukocyte can be modified using membrane-active enzymes such as phospholipase A. These findings support the suggestion that chemotactic factors interact with the phospholipid bilayer of the cell membrane.

Human serum albumin (HSA) (Behringwerke, Marburg, Germany) was denatured either by treatment at *p*H 12 overnight at 20° C followed by return to *p*H 7, or by reduction-alkylation with 2-mercaptoethanol (0.1 M) and iodoacetamide (0.02 M). These preparations were dialysed against Gey's solution, *p*H 7.2 for use and the degree of denaturation achieved was measured by difference spectroscopy against native HSA. Butyryl HSA and guanidyl HSA were prepared as described previously[2]. Milk proteins including fractionated samples of

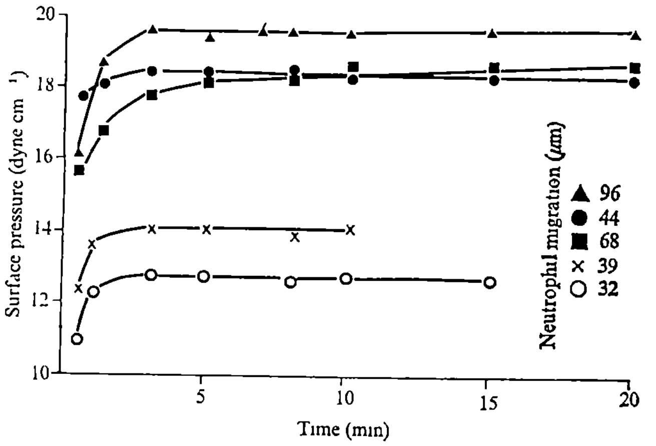

Fig. 1 Surface activity and chemotactic activity for neutrophils of native and modified human serum albumin (1 mg ml^{-1}). Both activities were measured on the same sample of each protein on the same day. The graph shows surface pressure measured against time after formation of the surface. The figures on the right represent the migration of neutrophils through a filter of 3 μm pore size towards each of the proteins, measured as the distance reached by the leading front of cells after 75 min at 37° C. ▲, Alkali-denatured HSA, ■, butyryl HSA, ●, reduced alkylated HSA, ×, guanidyl HSA, ○, HSA

casein were obtained by courtesy of Dr R. L. J. Lyster, National Institute for Research in Dairying, Shinfield, Reading. Unfractionated 'Hammarsten' casein was purchased from Merck A.G., Darmstadt, Germany.

To obtain a direct comparison of the two activities in a physiological medium, surface pressure and chemotactic activity were both measured on the same sample of each protein at 1 mg ml^{-1} in Gey's solution. The surface pressure at the air/Gey's solution interface was calculated from measurements of the force required to pull a glass plate out of the protein solution or Gey's solution at 20° C at timed intervals after pouring. Neutrophil leukocytes were obtained from normal human blood. The chemotactic assay was performed using modified Boyden chambers as described previously[2] and leukocyte migration within the micropore filter was measured by the leading front method of Zigmond and Hirsch[5]. The experimental conditions usually employed in Boyden-type assays do not distinguish directional from nondirectional locomotion of leukocytes. But using the modified assay systems described by Zigmond and Hirsch[5], the proteins studied here have previously been shown[6] to enhance both directional and nondirectional locomotion of leukocytes and may therefore legitimately be described as chemotactic factors.

Figures 1 and 2 show the surface pressures of various protein preparations and the migration of neutrophils towards the same preparations. Procedures which increased the surface activity of HSA (butyrylation, alkali denaturation) yielded products which attracted leukocytes more strongly than those showing smaller changes in surface activity, for example guanidyl-HSA (Fig 1).

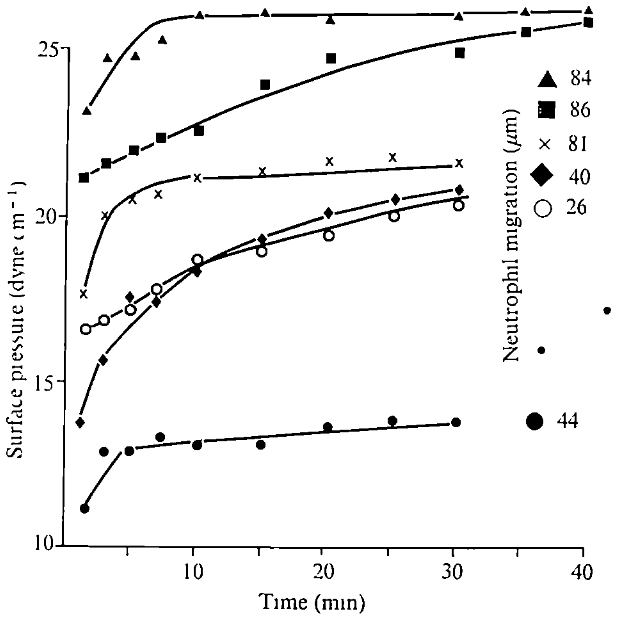

Fig. 2 Surface activity and chemotactic activity for neutrophils of fractionated milk proteins (each protein at 1 mg ml^{-1}). Both activities were measured on the same sample of each protein on the same day. Further details as in the legend to Fig 1. ▲, β-casein, ■, whole casein, ×, α-casein, ◆, α-lactalbumin, ○, k-casein, ●, β-lactoglobulin

The data for milk proteins are shown in Fig 2. Again the proteins (β-casein, α-casein and whole casein) which were most surface active also attracted neutrophils most strongly. Chemotactic substances may therefore be surface active. It is, however, important to note that not all surface active substances are chemotactic. Although the bee venom peptide, melittin, is highly surface active[7], it does not attract leukocytes. Cells incubated with melittin (Sigma, 10^{-5} M to 10^{-6} M) and observed directly showed cytoplasmic movements for a few minutes and

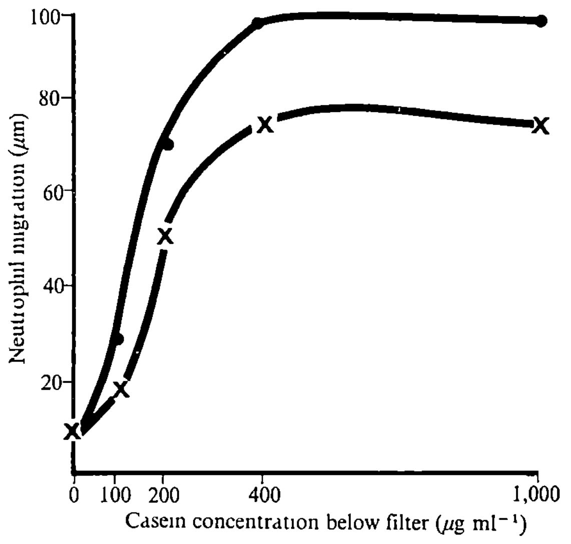

Fig. 3 Effect of phospholipase A on the migration of human neutrophils towards varying concentrations of whole casein. Healthy, freshly taken neutrophils (1×10^6 ml^{-1}) were preincubated for 1 h at 37° C with whole casein (50 μg ml^{-1}) with or without bee venom phospholipase A (50 mU ml^{-1} purified by Dr R. A. Shipolini, Department of Chemistry, University College London[8]). The cells and proteins were then added to the upper compartment of a Boyden chamber. The cells were allowed to migrate for 75 min towards casein at varying concentrations with (●) or without (×) phospholipase A (50 mU ml^{-1}) in the lower compartment. The phospholipase A alone had no effect on leukocyte migration. Provided that the neutrophils had not been spun in Ficoll-triosil, and that they were preincubated with the chemotactic factor as well as phospholipase, the result shown was reproducible.

then flattened out and sometimes lysed At lower, nontoxic, concentrations there was no effect on locomotion Chemotactic proteins probably require to interact with the cell membrane to initiate a locomotor response but without causing sufficient perturbation to damage the cell Furthermore, small surface active molecules such as the nonionic detergent, Tween 80 (Atlas, Wilmington, Delaware) or the anionic detergent sodium dodecyl sulphate showed no chemotactic activity at noncytotoxic concentrations, suggesting that chemotactic factors may require to be macromolecules

The hypothesis that chemotactic proteins interact with the membrane bilayer is supported by preliminary studies of the influence of treatment with membrane active enzymes on the migration of leukocytes towards the substances discussed above Treatment with phospholipase A enhanced the migration of neutrophils towards these substances (Fig 3) This effect was observed using a highly purified enzyme from bee venom[8] as well as with commercial preparations of phospholipase A derived from the venom of Russell viper (Koch-Light) and *Naja naja* (Koch-Light) To interact with the phospholipids of the cell membrane, phospholipase A has been shown to require the presence of direct lytic factor which is not present in pure enzyme preparations But surface active agents[9] and polypeptides with nonpolar groups[10] may replace direct lytic factor and allow the phospholipase to reach its substrate It is possible that chemotactic factors might act in the same way Glycosidases (neuraminidase, β-glucosidase, β-galactosidase) had little effect on leukocyte migration Trypsin at high doses (2 mg ml^{-1}) caused some reduction in migration

These results suggest that the locomotor response of leukocytes to chemotactic factors is initiated by an interaction of the factor within the cell membrane bilayer itself, rather than with superficially placed structures, and that the relatively hydrophobic nature of these factors allows them to penetrate to interact at hydrophobic sites within the bilayer This is supported by the enhancement of their chemotactic activity by phospholipase A which may facilitate entry of the chemotactic factor to the interior of the membrane Interaction with a site deep in the membrane may be an essential preliminary step in initiating intracellular events such as microfilament contraction leading to locomotion Since it has also been suggested that the ability of particles to be phagocytosed by neutrophils and macrophages may be determined by the relative hydrophobicities of the particle and of the cell which is to ingest it[11,12], it is possible that phagocytosis as well as locomotion is initiated by an interaction similar to that proposed here

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B cell-derived immunoglobulin on activated mouse T lymphocytes

IT is generally accepted that B lymphocytes express a high density of surface immunoglobulin (Ig) The presence of surface Ig on T lymphocytes, by contrast, is controversial Thus, in mice, T cell surface Ig is considered to be undetectable by some workers[1–3], others, by contrast, report that T lymphocytes express as much surface Ig as B cells[4]

Several groups have shown surface Ig on activated T cells (T-TDL) obtained from thoracic duct lymph of irradiated F_1 hybrid mice injected with parental strain thymus cells[4–6] This communication presents evidence that a proportion of this Ig is of B cell origin

T-TDL were collected from irradiated (800 r) (CBA×C57BL)F_1 hybrid mice injected 4–6 d previously with 2×10^8 CBA or C57BL thymus cells[7] Surface Ig on T-TDL was investigated by indirect immunofluorescence[8] When T-TDL were collected 4–5 d after thymus cell injection, 49% were stained by purified rabbit anti-mouse Ig antibodies (Table 1) The staining was clearly discernible but was less intense than that shown by a small subpopulation (<0 3%) of the cells, the latter have been shown by other criteria to be B cells[9] Most of the Ig detected on T-TDL collected at this stage was IgM Staining with antisera against IgG$_1$, IgG$_{2a}$ and IgG$_{2b}$ was not above the control levels (3%) obtained with normal rabbit IgG With T-TDL collected at 5–6 d, a higher proportion of the cells was Ig-positive (Table 1) and the intensity of staining was increased IgM continued to be the predominant class, although most of the cells also expressed IgG

The great majority of Ig-positive cells in T-TDL were undoubtedly T cells (95% are θ-positive) The surface Ig on on T-TDL could have been synthesised by these cells, alternatively, it might have been derived from B cells, for example, those present in the thymocyte inocula from which the T-TDL were derived To investigate this possibility, T-TDL were prepared from thymocytes depleted of B cells by passage through a Sephadex G-100 column conjugated with rabbit anti-mouse Ig antibody (modified from ref 10) This procedure caused a 12-fold reduction in numbers of strongly Ig-positive (B) cells, that is, from 0 9% to 0 08% No loss of B cells occurred when thymus cells were passed through control columns coated with normal rabbit IgG (NRIgG)

T-TDL derived from thymus cells filtered through a control column showed no changes in Ig staining relative to T-TDL derived from untreated thymocytes (see Tables 1 and 2) There was, however, a marked decrease in Ig-positive cells when the thymus cell precursors of T-TDL were passed through the anti-Ig immunoadsorbent column (Table 2) A higher proportion of T-TDL derived from B cell-depleted thymus were stained after incubation with NRIgG (Tables 2 and 3)

T-TDL depleted of surface Ig were still capable of specific antigen recognition Thus, they were unimpaired in their ability to lyse ^{51}Cr-labelled allogeneic tumour cells during a 6 h incubation period (Table 3) The presence of purified rabbit anti-mouse Ig antibody at a concentration of 1 mg ml^{-1} did not significantly impair lysis (Table 3)

The above experiments clearly suggested that much of the IgM on T-TDL was derived from B cells in the thymus cell inocula used to prepare the cells Direct evidence on the origin of the IgG on T-TDL was obtained with the use of an antiserum directed against allotype *a* of the Ig-1 locus expressed by IgG$_1$ and IgG$_{2a}$ (G F Mitchell and L Herzenberg, personal communication) (see legend to Fig 1)

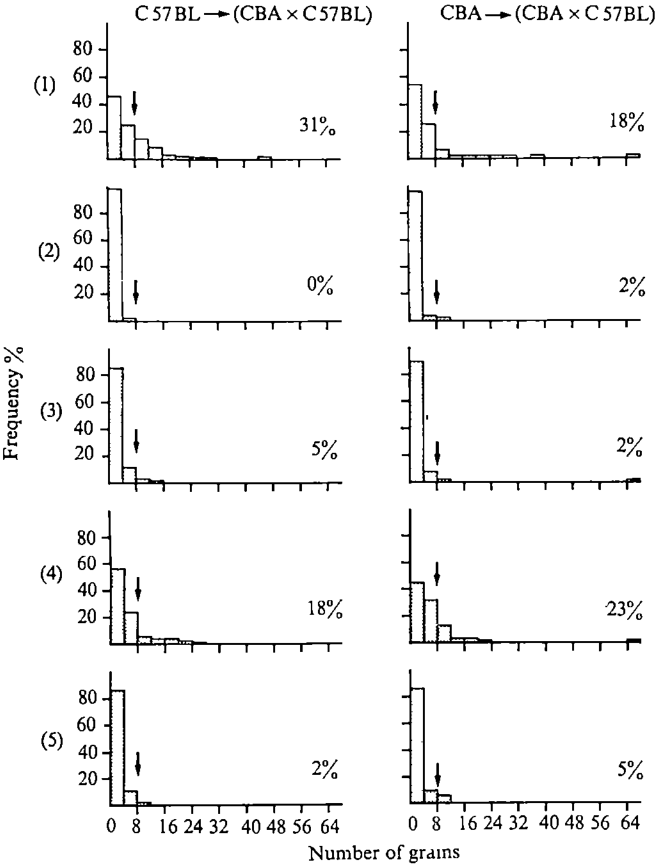

Fig. 1 T-TDL were generated by the transfer of 2×10^8 C57BL or CBA thymocytes in irradiated (800 r) (C57BL×CBA)F$_1$ hybrids T-TDL were collected 5–6 d after transfer Aliquots of 10^7 CBA- or C57BL-derived T-TDL were incubated with one of the following (1) 200 µg of ^{125}iodine-labelled *b* anti-*a* allotype γ-globulin (^{125}I-*b* anti-*a* Ig), (2) uniodinated *b* anti-*a* Ig in a 5-fold excess for 30 min, and then 200 µg of iodinated ^{125}I-*b* anti-*a* Ig, (3) a 5-fold excess of CBA γ-globulin (*a* Ig) in the presence of 200 µg iodinated ^{125}I-*b* anti-*a* Ig, (4) a 5-fold excess of C57BL γ-globulin (*b* Ig) in the presence of 200 µg iodinated ^{125}I-*b* anti-*a* Ig, (5) 200 µg of ^{125}iodine-labelled normal C57BL γ-globulin (^{125}I-*b* Ig) All incubations were at 4° C for 30 min, cells were then freed of unbound γ-globulin by washing through discontinuous gradients of foetal bovine serum, and finally processed for autoradiography After development, grain counts were performed on four coded slides per group, a minimum of 200 cells per group were counted The percentage of cells bearing more than eight grains (indicated ↓) is shown in each group

T-TDL bound this anti-*a* allotype serum irrespective of whether they were derived from CBA (allotype *a*) or C57BL (allotype *b*) thymus (Fig 1) The binding of the labelled antibody was specific since it was blocked by pretreatment with a five-fold excess on non-iodinated antibody and was reduced by the presence of CBA but not C57BL γ-globulin (Fig 1), furthermore, it stained B cells from lymph nodes of CBA mice but not of C57BL mice (data not shown) These studies indicated that, in the case of T-TDL derived from C57BL thymus, part of the IgG detected on the cells was of recipient origin

The demonstration of surface immunoglobulin on T-TDL by indirect immunofluorescence (Table 1) is in accord with the published data of other groups[4–6] It is probable that all T-TDL carry some surface Ig since staining using purified anti-Ig antibodies detected over twice as many Ig-positive blasts as an IgG fraction of the anti- κ serum (Table 1)

The data presented here suggest that the surface IgM, detected by immunofluorescence, was derived from donor B cells in the thymus cell suspensions from which the T-TDL were prepared

The IgM on T cells might be adsorbed by the Fc binding site, this would be consistent with evidence that a high proportion of H2-activated T cells (ATC) from spleen form rosettes with sheep erythrocytes (SRBC) coated with anti-SRBC (an Fc dependent process)[12,13] If this were the sole mechanism, however, it would be paradoxical that T-TDL prepared from B-cell-depleted thymus cells showed decreased surface IgM, that is, one might have expected recipient Ig to replace the missing donor Ig Significantly rosette formation by ATC from spleen was blocked by free IgG but not IgM Thus if IgM is not cytophilic for T blasts, how are we to account for its presence on T-TDL? An intriguing possibility, analogous to that proposed by Crone *et al* [3], is that the donor IgM antibody might be bound not only to an Fc receptor but also by its combining site to soluble alloantigen specifically captured by the endogenous T cell receptor According to this view, donor B cells in the thymus inoculum would produce antibody against the recipient alloantigens, some of the antibody would bind to antigen already captured by the antigen-specific receptors on T-TDL, binding would be stabilised by the attachment of the antibody to the Fc receptor Removal of donor B cells would thus remove the precursors of cells producing such antibody This mechanism might explain the specific qualities of the T-TDL surface IgM isolated by Cone *et al* [14]

The IgG of host origin cannot be accounted for by this

Table 1 Immunoglobulin subclasses on normal T-TDL

T-TDL stained with	% Fluorescent staining (± s e) Day 4/5*	Day 5/6†
Anti-IgA	9±3	7
Anti-IgM	34±4	78
Anti-IgG$_1$	4±2	55
Anti IgG$_{2a}$	0 7±0 7	8
Anti-IgG$_2$	0 3±0 3	12
Anti-κ light chains	23±3	42
Anti-immunoglobulin antibodies	49±8	55
Normal rabbit IgG	3±1	10

* Number of days after thymus cell transfer, mean ± s e of three experiments

† Results averaged from two experiments, duplicates within 13% of the mean

2×10^8 CBA thymocytes were transferred to 800 r irradiated (CBA×C57BL)F$_1$ hybrid mice T-TDL were collected 4/5 d after thymocyte transfer[7] and were thoroughly washed with HEPES buffered Eagle's medium Aliquots of 10^7 T-TDL were incubated for 30 min at 4° C with 100 µg of the IgG fraction of various rabbit anti-mouse immunoglobulins[8] Bound antibody was detected by a fluorescein-labelled goat anti-rabbit Fc serum Cells were examined in suspension under a Leitz incident light ultraviolet microscope, and the percentage of positive cells determined for each coded preparation A minimum of 200 cells were counted per preparation[8] It is to be emphasised that the specificity of the antisera has already been established[20] They were titrated for specific immunofluorescence against lymph node and spleen cells Furthermore, the staining seen on blasts with NRIgG was never seen on small lymphocytes

Table 2 Staining of T-TDL after anti-Ig column pretreatment of progenitor thymocytes

Column	T-TDL staining with	% Fluorescent staining (± s e) Day 4/5*	Day 5/6†
Anti-Ig	Anti-Ig antibodies	12± 3	29
	IgG anti-IgM	15±11	ND
	NRIgG	7± 3	13
NRIgG	Anti-Ig antibodies	49±12	69
	IgG anti-IgM	62±27	ND
	NRIgG	8± 1	4

* Number of days after thymus cell transfer, mean ± s e of three experiments

† Results averaged from two experiments, duplicates within 10% of the mean

Aliquots of 10^7 CBA thymocytes were filtered through Sephadex G-100 columns to which rabbit anti-mouse Ig antibody or NRIgG had been covalently attached[10] 2×10^8 of these filtered cells were transferred to 800 r irradiated (CBA×C57BL)F$_1$ hybrids, and the T-TDL were collected 4/5 or 5/6 d after transfer T-TDL were thoroughly washed with HEPES buffered Eagle's medium and stained for surface immunoglobulin as described in Table 1

Table 3 Release of ^{51}Cr from DBA/2 mastocytoma cells by DBA/2-activated CBA T-TDL

Treatment of T-TDL cells	% Fluorescent staining of T-TDL with anti-Ig antibody	NRIgG	Antibody in culture	% ^{51}Cr release Exp 1*	Exp 2†
Filtered through NRIgG column	69	8	none	94	59
			anti-Ig	87	55
			NRIgG	93	61
Filtered through anti-Ig column	16	25	none	94	62
			anti-Ig	85	60
			NRIgG	88	64
Spontaneous release from tumour cells				19	9

* Killer target ratio 20 1

† Killer target ratio 10 1

CBA thymocytes were filtered through anti-Ig or NRIgG immunoadsorbent columns 2×10^8 of these cells were transferred to 800 r irradiated (CBA×DBA/2)F$_1$ hybrids T-TDL were collected 5/6 d after thymocyte transfer, and after washing, were mixed with ^{51}Cr-labelled P815Y mastocytoma cells at a ratio of 20 1 and 10 1[11] The cell mixtures were incubated at 37° C for 6 h and the % ^{51}Cr release then counted In addition, 1 mg ml^{-1} of rabbit anti-mouse Ig antibody or NRIgG was added to identical mixtures throughout the incubation period

mechanism since the F$_1$ (recipient) cells presumably cannot recognise the donor (parental) antigens This IgG, but not donor-derived IgG, is therefore probably acquired by Fc receptors It has not yet been possible, however, to obtain direct evidence that Fc receptors are expressed by T-TDL[9]

How can the discrepancy between these findings with ATC-spleen and T-TDL be explained? According to the model considered above, alloantigen bound to endogenous T cell receptors could act as an immunadsorbent to facilitate the capture of specific antibody, the Fc portion of this antibody might preferentially come to occupy the Fc receptors on the cell, thus saturating these receptors and preventing nonspecific uptake of extraneous Ig It is possible that the splenic architecture may allow T blasts (ATC) to be formed in an environment relatively free of alloantibody, for example in the T-dependent areas ATC collected from the spleen would therefore have free Fc receptors Subsequent migration of these cells through alloantibody producing areas, for example B-dependent areas, before their eventual appearance in the lymph, would saturate the Fc receptors with alloantibody It would follow, therefore, that Fc receptor blockade would be less evident on T-TDL derived from B cell-depleted thymus cells Though this prediction has yet to be verified, it is consistent with the finding that, unlike normal T-TDL, a high proportion of T-TDL derived from B cell-depleted thymocytes bound NRIgG (Tables 2 and 3)

The mechanism proposed for specific antigen and antibody capture by activated T cells can be invoked to explain two phenomena observed by other workers

(1) T rosette-forming cells (T-RFC) to SRBC from mice can, unlike most other T-cell reactions, be blocked by anti-light chain and anti-IgM sera It is suggested that such immune rosettes are formed by specifically activated T cells binding a complex of antigen and specific IgM antibody, the Fc of the immunoglobulin stabilising the binding of antigen and the free combining sites of the antibody being available to bind the RBC used to form rosettes[15]

(2) Bursectomised chickens are deficient in cells forming specific rosettes with SRBC But preinjection of these birds with an anti-SRBC serum restores the numbers of RFC to the levels found in normal chickens[16] It is possible that the antiserum used here contained antigen–antibody complexes in antibody excess If so, then according to the model considered above, the complexes would bind only to specific T cells the antigen attaching to the endogenous receptors of the cells and being held there by Fc binding of the antibody The free binding sites of the antibody would be available to form rosettes

The possible role of this mechanism in initiating or aiding cellular cooperation *in vivo* has been recently reviewed by Playfair[17]

Despite the evidence presented here and in other studies[18,19] that much of the surface Ig on T cells is of exogenous origin, the proposed mechanism for stabilising antigen-binding by T cells requires that these cells express antigen-specific receptors These may or may not be immunoglobulin But it is clear that in all experiments attempting to demonstrate endogenous synthesis of immunoglobulin by T cells, one must consider the possible presence of B cell-derived immunoglobulin

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Note added in proof As a corollary to the above model, if activated T cells are generated in situations where alloantibody is not produced, as for example between mice differing only at the M locus, immunoglobulin should not be demonstrable on the surface of the activated cells This has been shown experimentally to be the case (Hudson, L, Sprent, J and von Boehmer, H, unpublished data)

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Removal of specific cooperative T cell factor by anti-H-2 but not by anti-Ig sera

ANTIBODY production to thymus-dependent antigens requires cooperation between thymus derived (T) and bone marrow derived (B) lymphocytes[1] B cells are the precursors of the antibody producing cells, but they apparently require the stimulatory influence of T cells in order to be triggered to proliferate and differentiate into plasma cells The nature of the cooperative stimulus which passes from T cell to B cell is not yet established, though several possibilities have been discussed[1–3] There is evidence that T cells produce various mediators which may stimulate B cells, including nonspecific as well as antigen-specific T cell products

One of us has recently described the production of an antigen-specific factor by specifically activated or 'educated' mouse T cells[4] The factor is capable of replacing T cells in a cooperation dependent antibody response *in vivo* We have attempted to identify this T cell product by removing it with various antisera immobilised as immunoadsorbents The results described here show that the factor could not be removed by a sheep anti-mouse immunoglobulin (Ig) adsorbent, but was removed completely by congenically raised anti-H-2 antibodies

The production and activity of the specific cooperative T cell factor have been described in detail in a previous report[4] The antigen used was the multichain synthetic polypeptide poly(Tyr, Glu)-polyDLAla—polyLys, abbreviated as (T,G)-A—L (ref 5), supplied by Dr Edna Mozes (Department of Chemical Immonology, The Weizmann Institute of Science, Rehovot, Israel) Mice used were of the strains BALB/c, C57BL/10ScSn (B10) and strains congenic with B10, namely B10 D2, B10 Br and B10 A All strains were obtained from the Laboratory Animal Centre, Carshalton, and were bred at the Department of Pathology, Tennis Court Road, Cambridge In brief, T cells, primed or educated to (T,G)-A—L *in vivo*, were incubated together with antigen *in vitro* for 6–8 h The cells were then removed by centrifugation and the supernatant, containing the T cell factor, transferred together with bone marrow cells and (T,G)-A—L into lethally irradiated, syngeneic recipients After 14 d the direct plaque forming cell (PFC) response to (T,G)-A—L in the spleens of the recipients was measured and compared with controls receiving B cells and antigen but no factor, or B cells with T cells and antigen

Table 1 Inability to remove T cell factor by anti-mouse Ig adsorbent

Cells and factors transferred	Antigen	Mean* PFC per spleen
B cells†	(T, G)-A—L‡	1 699
B cells + T cells§	(T, G)-A—L	3 805
B cells + T cell factor¶	(T, G)-A—L	3 820
B cells + T cell factor after adsorption by		
(1) normal sheep globulin	(T, G)-A—L	3 778
(2) sheep anti-mouse Fab′	(T, G)-A—L	3 792
Standard error		0 176

* $\log_{10}$ geometric means
† 10^7 bone marrow cells
‡ 10 μg in solution
§ 10^8 normal thymocytes
¶ Produced by one spleen equivalent educated T cells

Direct anti-(T, G)-A—L PFC responses in irradiated (750 r) BALB/c mice 14 d after receiving combinations of syngeneic B cells, T cells and T cell factor, adsorbed as indicated

Antisera used for absorption of the factor were raised as follows Sheep anti-mouse Fab′ was prepared by immunisation with the Fab′ fragment of BALB/c myeloma protein AdjPc5 (the myeloma was originally provided by Dr Michael Potter) First inoculation was given in Freund's complete adjuvant, followed by several inoculations in solution The alloantisera used were anti-H-2^d, anti-H-2^k and anti-H-2^b and were raised in strains congenic with those producing the factor under test Anti–H-2^d was prepared by repeated weekly inoculation of DBA/2 spleen cells into B10 Br (H–2^k) mice, anti-H-2^k was prepared by repeated weekly inoculation of CBA spleen cells into B10 D2 (H-2^d) mice, and anti-H-2^b by repeated weekly inoculation of either C3H WB or B10 spleen cells into B10 A (H-2^a) mice All the sera were cytotoxic, in the presence of complement, for lymphocytes of the appropriate H-2 type, with the exception of one of the anti-H-2^b sera (B10 cells into B10 Br) Immunoadsorbents were prepared from these sera, as well as from normal mouse serum, by precipitating the gamma globulin with 45% $(NH_4)_2SO_4$ and coupling to activated sepharose after removal of the $(NH_4)_2SO_4$[6] T cell factor was tested immediately after preparation by passage over the immunoadsorbents in the form of short columns, and was then mixed with B cells and (T,G)-A—L and transferred into irradiated recipients as above

Table 2 Removal of T cell factor by anti-H-2 adsorbent

Cells and factors transferred	Antigen	Mean* PFC per spleen
B cells†	(T, G)-A—L‡	2 176
B cells + T cells§	(T, G)-A—L	4 212
B cells + T cell factor¶	(T, G)-A—L	4 107
B cells + T cell factor after adsorption by		
(1) normal mouse globulin	(T, G)-A—L	4 130
(2) anti-H-2^d	(T, G)-A—L	2 220
(3) anti-H-2^k	(T, G)-A—L	4 198
Standard error		0 145

* $\log_{10}$ geometric means
† 10^7 bone marrow cells
‡ 10 μg in solution
§ 10^8 normal thymocytes
¶ Produced by one spleen equivalent educated T cells

Direct anti-(T, G)-A—L PFC responses in irradiated (850 r) B10 D2 (H-2^d) mice 14 d after receiving combinations of syngeneic B cells, T cells and T cell factor The factor, produced by B10 D2 T cells, was adsorbed as shown

The first results show an attempt to remove the T cell factor by passage over a sheep anti-mouse Fab′ column (Table 1) The factor, which was in this case prepared from BALB/c T cells educated to (T,G)-A—L, emerged from such a column with undiminished ability to cooperate with B cells in the response to (T,G)-A—L *in vivo* The ability of the anti-Fab′ column to bind mouse Ig was established using radiolabelled mouse Ig The conclusion may be drawn from this experiment that the nature of the cooperative T cell factor is not that of conventional mouse Ig, at least in so far as it does not seem to carry mouse κ chains, the predominant light chain type of mouse Ig But since the sheep serum was raised against a mouse myeloma Fab′, various other possibilities still remain open, though they are rather unlikely, for example the T cell factor carries λ rather than κ chains, or is composed of heavy chains without light chains With these reservations, and within the limitation that only one anti-mouse Ig serum has been used in this work so far, the result shown in Table 1 strongly suggests that T cell factor is not Ig This finding also rules out any remaining suspicion that the T cell factor may be a B cell contaminant

Attempts were then made to remove the T cell factor, with immunoadsorbents prepared from alloantisera Congenic strains derived from the B10 genotype and differing only in the major histocompatibility locus, H–2, were used Table 2 shows the results of an experiment in which T cell factor was prepared in the B10 D2 strain (H–2^d) and passed through immunoadsorbents of either anti-H-2^d or anti-H-2^k specificity, or normal mouse globulin The factor was totally removed by the anti–H–2^d adsorbent, but not at all by anti–H–2^k or normal mouse globulin Since the anti-H-2^d serum was prepared in a congenic strain (by injection of DBA/2 spleen cells into B10 Br mice), it is very unlikely to have reacted with any B10 D2 antigens other than those in the H-2 complex The result thus implies that the T cell factor carries alloantigenic determinants of the T cells from which it is produced This finding was confirmed in another strain, B10 (H–2^b) (Table 3) T cell factor was prepared from B10 T cells educated to (T,G)-A—L, and passed through adsorbents containing anti-H-2^{b} or anti-H-2^{d} Two anti-H-2^{b} adsorbents were employed, both prepared from alloantisera raised in the congenic B10 A (H-2^{a}) strain, one by inoculation of C3H WB spleen cells and the other by inoculation of B10 spleen cells themselves Both these adsorbents completely removed the T cell factor produced by B10 educated T cells In contrast, the anti-H-2^{d} adsorbent which successfully removed the factor produced by B10 D2 T cells did not adsorb that produced by B10 T cells (Table 3) It may be noted that the latter result rules out the possibility that the anti-H-2 sera adsorb the factor because they contain antibodies cross-reacting with (T,G)-A—L itself, to which the factor may be bound[7] An anti-(T,G)-A—L adsorbent itself also fails to remove the factor (personal observation)

Table 3 Removal of T cell factor by anti-H-2 adsorbent

Cells and factors transferred	Antigen	Mean* PFC per spleen
B cells†	(T, G)-A—L‡	2 133
B cells + T cell factor§	(T, G)-A—L	4 170
B cells + T cell factor after adsorption by		
(1) anti-H-2^{b}¶	(T, G)-A—L	2 176
(2) anti-H-2^{b}‖	(T, G)-A—L	2 112
(3) anti-H-2^{d}	(T, G)-A—L	4 225
Standard error		0 134

* log $_{10}$ geometric means
† 10^{7} bone marrow cells
‡ 10 µg in solution
§ produced by one spleen equivalent educated T cells
¶ C3H WB spleen cells inoculated into B10 A mice
‖ B10 spleen cells inoculated into B10 A mice

Direct anti-(T, G)-A—L PFC responses in irradiated B10 (H-2^{b}) mice 14 d after receiving combinations of B cells and T cell factor The factor, produced by B10 T cells, was adsorbed as shown

These results strongly suggest that the cooperative T cell factor is physically associated with alloantigens of the strain in which it is produced The interpretation of these findings clearly depends on the specificities of the antibodies present in the alloantisera used The alloantigens coded for by the H-2 region and expressed on the T lymphocyte surface include the products of the H-2K and H-2D regions, and in addition probably the products of the H-2-linked immune response (Ir) genes[8] The latter control the levels of immune responses to specific antigens and in the mouse have been mapped in a region within the H-2 locus, between the H-2K and H-2D 'ends' and closer to H-2K[8] There is evidence that Ir genes are functionally expressed in both T[8,9] and B cells (refs 10-12 and G M Shearer, pages 38-39 in ref 8) The finding that antisera to the H-2 complex remove the T cell factor may imply either that the factor is identical with the product of the H-2K or H-2D genes, or that it is an *Ir* gene product, or possibly that it is some as yet unidentified molecule which becomes physically attached to one of the H-2 products Perhaps the most attractive speculation is that the factor consists of both the Ir and H-2K or H-2D gene products physically combined The products of H-2K or H-2D might provide the 'constant' region of a molecule analogous to immunoglobulin, with Ir gene products providing the variation required for specific antigen binding The use of recombinant strains, providing different combinations of H-2K, Ir and H-2D, should resolve this problem

What is the relationship between T cell factor and T cell receptor? The most important feature of the T cell factor is its specificity for the inducing antigen[4] By analogy with B cells, it is highly unlikely that T cells would produce a specific molecule that was different, in terms of antigen recognition, from the T cell receptor It seems most probable, therefore, that the T cell factor is the soluble expression of the T cell receptor, that is, that the T cell factor and the T cell receptor are closely related molecules The conclusion that the T cell receptor is intimately associated with H-2 region products finds considerable support in the work of others Attempts to demonstrate Ig on the T cell surface have been largely unsuccessful (refs 13, 14, E Unanue, pages 207-220 in ref 8, and J W Uhr, pages 228-238 in ref 8), despite some positive reports[15-18] Anti-Ig serum fails to inhibit T cell functions such as mixed lymphocyte reaction (MLR) (I Roitt, quoted in ref 1, page 118) and graft-*versus*-host (G*v*H) reaction[19], though antigen binding by T cells, helper activity and 'hot' antigen T cell 'suicide' have all been successfully inhibited by such sera[20-22], and a 7S IgM derived from activated T cells is able to cooperate with B cells *in vitro*[3] The likelihood that T cells adsorb antibody on to their surface[23] makes interpretation of the positive results difficult against the background of widespread failure to show conclusively that Ig is the primary T cell receptor The failure, reported here, to remove the T cell factor on an anti-Ig adsorbent also strongly suggests that the factor, and by inference the T cell receptor, are non-Ig in nature On the other hand, positive association between T cell receptor and histocompatibility (H) antigens includes the blocking of MLR and PPD proliferative responses of human lymphocytes by anti-HL-A sera *in vitro*[24,25], the inhibition of the G*v*H response by alloantibodies[19], and the abolition of the response of guinea pig T cells to specific antigens *in vitro* by antisera against H antigens[26] This last observation is clearly analogous to the absorption of T cell factor by alloantibodies, and it is interesting to note that in the guinea pig the active antibodies are apparently directed at H rather than Ir gene products[27] The authors, in this latter case, also conclude that H and Ir gene products must be linked physically

A summary of our conclusions from the observations described here is as follows H-2 region genes expressed by T cells are very probably responsible for antigen recognition by T cells After recognising antigen, T cells release a soluble, antigen-specific factor which, in conjunction with antigen, is apparently able to transmit a stimulatory signal to B cells The factor consists either wholly or partly of H-2 region gene products This further suggests that the latter are important in the interaction with the B cell and (or) perhaps the macrophage

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Isolation of a purified preparation of metabolically active retinal blood vessels

THE retinal vascular system has long been of interest to both clinicians and basic scientists because of its involvement in a number of systemic diseases, particularly hypertension and diabetes mellitus, and its accessibility to *in vivo* study through direct observation due to the transparency of the eye. This accessibility has made it an ideal system for the study of a portion of the microcirculation in various physiological and pathological states as well as under the influence of pharmacological agents. Such studies have advanced considerably the clinical and experimental investigation of retinal vascular disease which is one of the most common causes of visual disturbance and blindness in man. But *in vitro* study of retinal blood vessels has been restricted largely to the examination of retinal digest preparations obtained with the use of proteolytic enzymes[1] or by autolysis of the retina in water[2], and of isolated vessels obtained by microdissection techniques[3]. Metabolic studies on such preparations of isolated vessels have been extremely limited[3] because of the non-physiological conditions used or their tedious and time consuming nature. Therefore, we have developed a simple procedure for the isolation of a purified preparation of bovine retinal vessels which are metabolically active.

Retinal vessels were obtained from freshly enucleated bovine eyes obtained at a local slaughter house. Immediately after removal from the animal, the eyes were bisected and the retinae removed and placed in ice-cold buffer consisting of Earle's balanced salt solution (116 mM NaCl, 16 mM KCl, 1 mM $NaH_2PO_4 \cdot H_2O$, 0.8 mM $MgSO_4 \cdot 7H_2O$, 5.6 mM glucose, 1.8 mM $CaCl_2$) buffered with 28 mM HEPES (N-2-hydroxyethylpiperazine-N-2-ethane sulphonic acid (*p*H 7.4) and containing 0.01% bovine serum albumin, and 50 mg of penicillin G and streptomycin sulphate l^{-1}. The isolated retinae in buffer were carried back to the laboratory on ice where they were minced well with scissors and homogenised with 10 up and down strokes of a hand-held tapered Teflon pestle in a smooth glass tube. One part of tissue was homogenised in four parts of buffer (v/v). The homogenate was then poured over an 86 μm nylon sieve (Kressilk Products Inc., Monterey Park, California) and the sieve washed extensively with buffer. The material remaining on the sieve was washed off into a homogenisation tube and rehomogenised with five up and down strokes of the pestle. The resulting homogenate was again sieved through the nylon sieve and the sieve washed thoroughly with buffer. The material now on the sieve consisted of a purified preparation of retinal blood vessels which were almost devoid of non-vascular tissue contamination with the exception of trapped blood elements, as determined by phase contrast and electron microscopy as well as by light microscopic observations of fixed tissue sections. The yield of vessels from the retinae of 18 cow eyes was 281 mg of wet weight tissue. If preparations enriched in large or small diameter vessels were desired, these could be obtained by first sieving the rehomogenised material through a 210 μm nylon sieve followed by sieving of the filtrate on an 86 μm sieve. The material found on the 210 μm sieve contained vessels of large and medium diameter with attached and adhering vessels of smaller diameter whereas that on the 86 μm sieve consisted almost entirely of vessels of smaller diameter. These preparations were washed off the sieves into centrifuge tubes and sedimented at 1,085*g* for 2 min to obtain a pellet of vessels which were resuspended in a suitable volume of buffer for metabolic experiments.

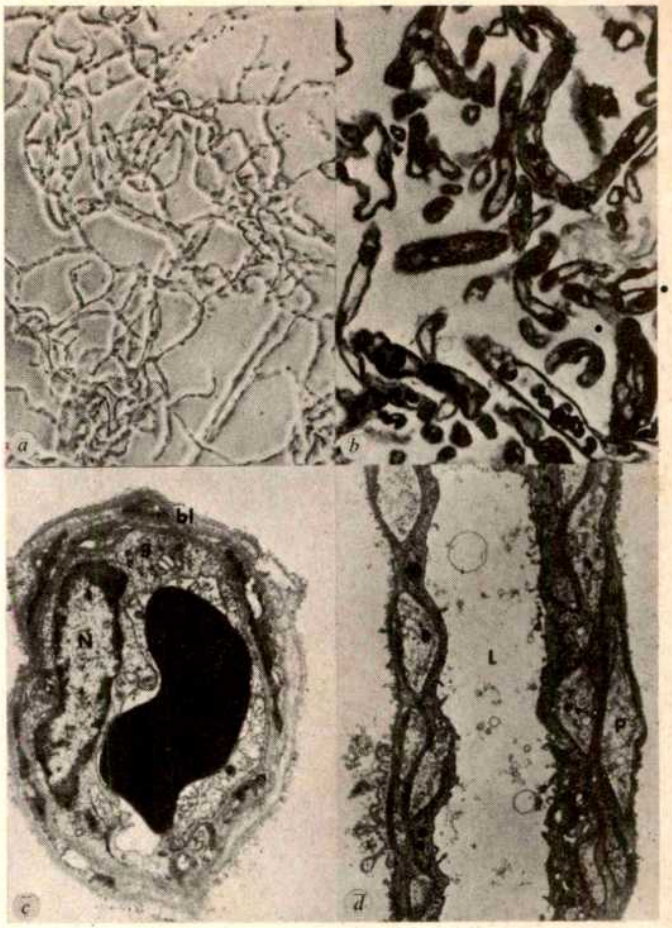

Fig. 1 *a*, Phase contrast photomicrograph of isolated bovine retinal vessel aggregate. Red blood cells are visible within vessel lumina. ×158. *b*, Photomicrograph of epoxy-embedded isolated bovine retinal vessel aggregate. Vessels are cut in various planes of section. Non-vascular surrounding tissues are virtually absent. Toluidine blue, ×470. *c*, Electron micrograph of cross section through isolated retinal vessel. A red blood cell occupies nearly the entire lumen. Endothelium is lined by basement lamina (bl) which surrounds cytoplasmic processes of adjacent pericytes. The endothelial cell exhibits a prominent nucleus (N) and Golgi apparatus (g). ×5447. *d*, Electron micrograph of longitudinal section through isolated retinal vessel. The lumen (L) of the vessel is devoid of cells. A thin endothelium (E) lines this vessel and is surrounded by basement lamina (bl) which varies greatly in thickness and also surrounds intramural pericytes (P) ×3062.

The metabolic activity of the vessel preparations was determined by incubating 0.5 ml aliquots of a suspension of vessels with shaking at 37° C in an atmosphere of oxygen in the presence of appropriate ^{14}C-labelled substrate in a $^{14}CO_2$ collection apparatus described elsewhere[4]. Radioactivity determinations were performed in a Beckman model LS-250 scintillation counter using a scintillation fluid containing one third Triton X-100 (ref. 5). Protein was determined by the method of Lowry[6]. ^{14}C-1-oleic acid (specific activity 9.1 mCi mmol^{-1}) and ^{14}C-2, 3-succinic acid (specific activity 18.3 mCi mmol^{-1}) were products of New England Nuclear Corporation (Boston, Mass.). ^{14}C-6-D-glucose (specific activity 10 mCi mmol^{-1}) was obtained from Calbiochem (Los Angeles, California).

Phase contrast microscopy was carried out on freshly isolated vessel preparations using a Zeiss RA or Ultraphot II microscope. For other microscopic examination isolated blood vessels were fixed for 1 h in cold Karnovsky's[7] fixative, buffered at *p*H 7.3. Postfixation was carried out in 2% OsO_4 buffered with 0.144 M sodium cacodylate. Tissues were dehydrated in a graded series of ethanols and propylene oxide followed by embedment in Epon-Araldite[8]. Thick (1 μm) and thin sections were cut with glass or diamond knives on a Porter-Blum-MT-2 Ultramicrotome. Thick sections were stained with toluidine blue for purposes of orientation and determination of vessel position. Thin sections were stained with uranyl acetate (5% in absolute ethanol) and lead citrate[9]. Light microscopic observations were made on a Zeiss Ultraphot II. Thin sections were observed with a Philips EM-300 electron microscope at original magnifications of 1,200–13,000.

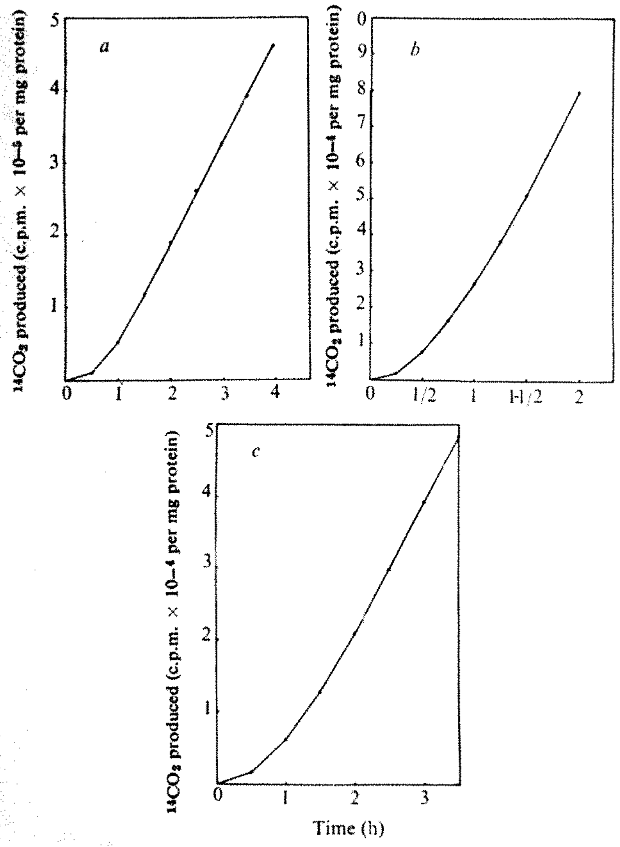

Fig. 2 Oxidative metabolism of several substrates by isolated retinal vessels: *a*, ^{14}C-6-D-glucose (0.25 μCi); *b*, ^{14}C-2,3-succinic acid (0.2 μCi); *c*, ^{14}C-1-oleic acid (0.2 μCi). Each incubation flask contained 83.5–236 μg of retinal vessel protein plus the designated amount of ^{14}C-labelled substrate in 0.51 ml of incubation buffer (minus glucose). Control experiments not shown in Fig. 2 include incubations with buffer alone and with bovine blood dilutions equivalent to blood elements entrapped in the corresponding vessel preparations (300→3,000 red blood cells). These controls yielded no significant $^{14}CO_2$ production over background from identical amounts of the above radioactive substrates.

The technique which we describe for the isolation of retinal blood vessels results in a loose pellet of tissue which to the naked eye appears as a twisted aggregation of very fine thread-like tubules. By phase contrast microscopy (Fig. 1*a*) the pellet can be shown to be composed of an extraordinarily clean preparation of isolated blood vessels. Although the vessels exhibit a wide range of diameters (7–50 μm), most of them are approximately 10 μm in diameter. A few red blood cells are seen free in the dispersion medium, but the remaining tissues are composed of clearly recognisable vessels with enclosed blood elements. The remarkable homogeneity of these tissues is more clearly demonstrated by conventional light microscopy (Fig. 1*b*). Sections 1 μm thick of fixed tissue reveal an aggregate of vessels cut in longitudinal, cross and oblique section. Measurements of these vessels compare favourably with those seen in phase microscopy.

Electron microscopic observations of isolated retinal vessels show an extremely clean preparation. Nonvascular tissues consist largely of blood cells, some of which are found free in the embedding medium. Cross sections of capillaries (Fig. 1*c*) exhibit red blood cells within their lumina. The vessel wall is composed of endothelial cells and a basement lamina which may split to enclose cytoplasmic extensions of smooth muscle cells or intramural pericytes. Endothelial cell nuclei are surrounded by a smooth contoured nuclear envelope interrupted by nuclear pores. Many acanthasomes and micropinocytotic vessels are seen in the peripheral cytoplasm, and a prominent Golgi zone is frequently noted in the perinuclear area. Endothelial flaps adjacent to cellular junctions are frequent modifications of the luminal plasmalemma. In longitudinal section (Fig. 1*d*) the morphological integrity of the endothelium is preserved. Numerous mitochondria lie within the endothelial cell cytoplasm.

The maintenance of the subcellular compartmentation of the vascular cells of preparations isolated using this procedure was supported by their ability to metabolise ^{14}C-labelled oleic and succinic acids and ^{14}C-glucose to $^{14}CO_2$ (Fig. 2), metabolic transformations which cannot be carried out by red blood cells. This was confirmed by control experiments run under identical conditions with amounts of blood elements equivalent to those found entrapped in the incubations with vessels. In the experiment with vessels after an initial lag period, the oxidation of the above substrates was maintained at linear rates and continued at these rates for several hours demonstrating the integrity of the Embden-Myerhof pathway, the pathway of fatty acid oxidation and the Krebs cycle in this preparation. Thus, morphologically and metabolically, this is an intact purified preparation of isolated small blood vessels obtainable in good yield and suitable for analytical, biochemical and pharmacological studies. The isolation procedure avoids the difficulties involved in previously described procedures for the isolation of retinal vessels involving trypsinisation, autolysis of the retina in water and microdissection. Most retinal vessel preparations isolated using these techniques have been used for morphological[1,2] and analytical[10,11] studies and almost nothing is known concerning their metabolism. Metabolic studies have been limited to histochemical investigations[12] and experiments using the Cartesian diver[3]. Investigations of the metabolism of small blood vessels from nonretinal tissues have also been limited to studies of preparations obtained by dissection. Such preparations can be derived readily in adequate amounts only from nonmammalian sources such as the swim bladder of the eel[13]. The availability of a procedure which enables the quick and simple isolation of a purified preparation of small blood vessels in good yield from mammalian retinas should permit further investigations of the properties of this important type of vascular tissue This procedure has also been modified for the isolation of blood vessels from bovine cerebral cortex[14] and may be adapted to the isolation of small blood vessels from other tissues

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Stimulation of ribosome synthesis during retarded ageing of human fibroblasts by hydrocortisone

CORTISONE[1] and hydrocortisone[2] can prolong the lifespan of human embryonic fibroblasts cultivated *in vitro* and this prolongation is accompanied by a significant increase in the saturation density The same effect has been observed in cultures of primary human amnion cells[3] One of the constant features during senescence *in vitro* of cells from different species is the progressive decrease of the cell density at which growth practically stops, and it has been suggested[4] that the two phenomena, ageing *in vitro* and decrease of the saturation density, are related

It is now well established that ribosome synthesis declines when division slows down during cell crowding[5–10] It has also been reported that RNA synthesis declines during cell senescence[11] Since it has been shown that hydrocortisone stimulates RNA synthesis[12–14] the increased saturation density and lifespan of cells carried in the presence of hydrocortisone could be due to a sustaining effect of the hormone on ribosome synthesis We show here that, in fact, human fibroblasts carried in hydrocortisone-supplemented medium incorporate more radioactive precursors into ribosomes and polyribosomes than the control cultures This effect may be correlated with the retarded ageing induced by the hormone *in vitro*

A human embryonic fibroblastic line was cultured with and without hydrocortisone The controls died at the 44th passage and the hydrocortisone treated cells at the 62nd passage At the 49th passage, cells carried in the hormone supplemented medium were subcultivated in medium without the hormone and died two passages later

At the 36th passage part of the cultures from each group were pooled, subcultivated and counted each day until the growth curves reached a plateau In addition, each day after subcultivation, cells from identical cultures were labelled and collected, and the cytoplasm extracts layered on 7–52% sucrose gradients (Fig 1) According to previous data[15] fractions 10–15 correspond to polyribosomes and fractions 16–25 to single ribosomes Significantly more ribosomes and polyribosomes were synthesised on day 1 and day 2 in hydrocortisone-treated cells During the third day, when the cultures exposed to hydrocortisone had already reached a plateau though the controls were still dividing, the radioactivity incorporated in the polyribosomal fractions was more or less identical in both groups (Fig 1) On day 4 the cultures in both groups had reached a plateau and as expected, very few polyribosomes were synthesised and the radioactivity incorporated in the ribo-

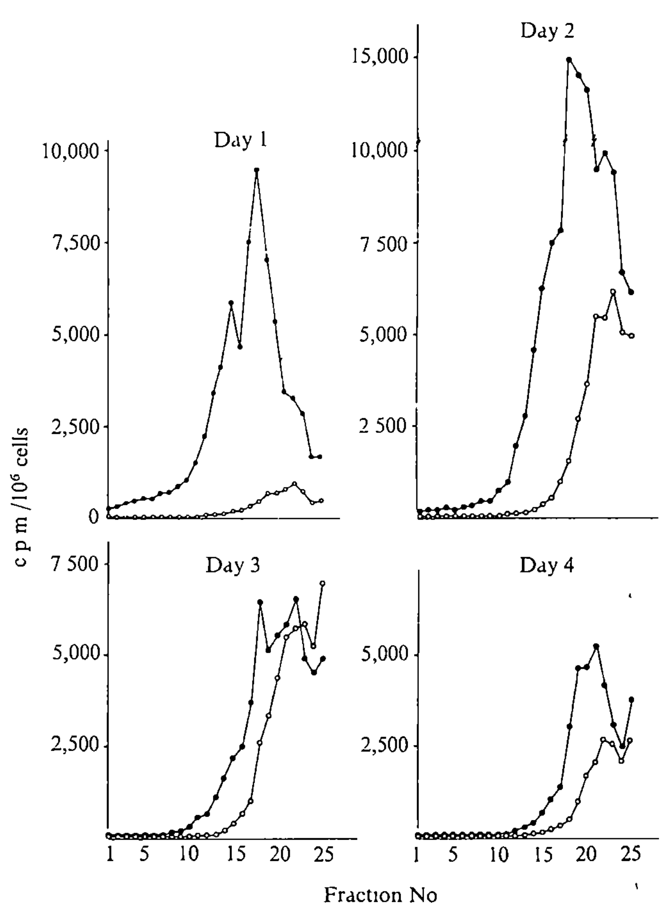

Fig 1 Incorporation of ^{3}H-uridine in ribosomes and polyribosomes each day after the 36th subcultivation of human fibroblasts carried with (●——●) and without (○——○) hydrocortisone Cultures were labelled with 5 μCi ml^{-1} ^{3}H-uridine (specific activity 10 Ci mmol^{-1}) for 3 h The cells were then scraped with a rubber policeman, washed twice in *p*H 8 6 buffer (0 01 M Tris, 0 14 M NaCl, 0 01 M MgCl$_2$) and treated for 10 min in the cold with 0 5% NP^{40} in buffer The cell suspensions were further treated with a Dounce homogeniser and examined under a microscope to see if nuclei were well separated from the cytoplasmas The cell suspensions were centrifuged at 20,000*g* for 15 min and the supernatants were layered on a 7–52% sucrose gradient which was centrifuged at 25,000 r p m for 3 5 h at 4° C in a SW 27 rotor The gradients were collected in 25 fractions and to each fraction 10% TCA was added The fractions were filtered through millipore filters which were digested in Instagel and the radioactivity counted in a liquid scintillation spectrometer The bottom of the gradient is on the left side of the abscissa

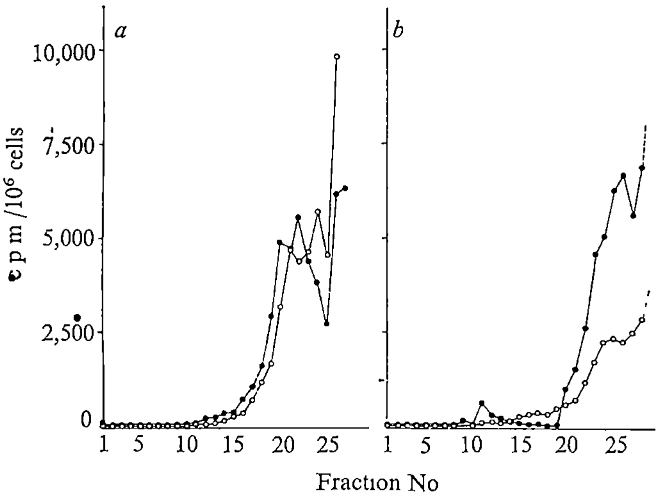

Fig 2 Incorporation of ^{3}H-uridine into ribosomes during *a*, the first and *b*, the second passage after withdrawal of the hormone (O——O) in cells that were cultured with hydrocortisone (●——●) beyond the lifespan of the controls Cells were labelled at the stationary phase and treated as described in Fig 1 The bottom of the gradient is on the left side of the abscissa

somal fraction had declined significantly in both groups Ribosomal synthesis, however, was higher in the hydrocortisone-treated cells No difference between the groups could be detected in the acid-soluble fractions, suggesting that the increased incorporation of uridine is not due to an increased penetration of the precursor into the cell

Since cells cultured in hydrocortisone beyond the lifespan of the controls died within two pasages when cultured in medium without the hormone, we decided to measure ^{3}H-uridine incorporation under these circumstances Figure 2 shows that there was no difference in the incorporation of ^{3}H-uridine into ribosomes from both groups during the first passage after withdrawal of the hormone A significant decrease, however, was found in the ribosomes synthesised after the second passage without the hormone To be sure that this peak corresponds to ribosomes, we separated the ribosomal subunits on a 5–20% sucrose gradient (Fig 3*a*) and then layered on a 7–52% sucrose gradient (Fig 3*b*) identical to the ones described in Fig 1 All subunits were found around fraction 20

Protein synthesis was also measured in hydrocortisone-treated cells and in the controls At the 36th passage cultures from each group were pooled and subcultivated separately Each day thereafter the cells were counted and labelled for 15 min with ^{14}C-labelled amino acids until the growth curves reached a plateau Acid-soluble and insoluble radioactivity were measured A significantly increased protein synthesis (Fig 4) was found on the first day after subcultivation in the cells carried in hydrocortisone supplemented medium When protein synthesis declined (due to cells reaching stationary phase) no difference could be seen in the two groups

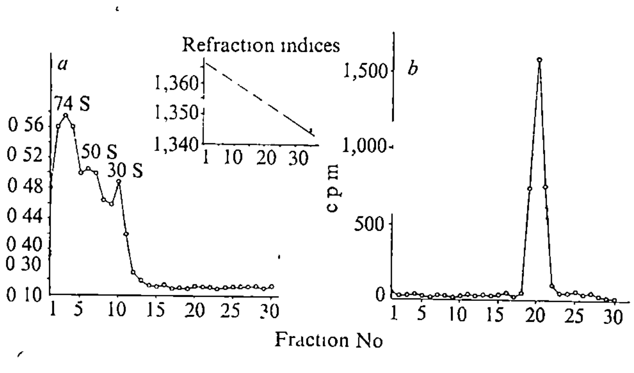

Fig 3 *a*, Separation of ribosomal subunits on a 5–20% sucrose gradient in control human fibroblasts Cells were labelled and the cytoplasmic fraction was prepared as described in Fig 1 and then treated with 0 2% sodium deoxycolate in buffer at 4° C for 30 min The suspension was then centrifuged for 2 h at 105,000*g* in a Beckman 40 rotor The precipitate was resuspended in 1 ml *p*H 7 45 buffer (0 0011 M Na phosphate, 0 0004 M $MgCl_2$) and layered on a 5–20% sucrose gradient which was centrifuged for 2 5 h at 25,000 r p m in a SW 27 rotor The absorbance of the fractions collected from the gradient was measured at 260 nm *b*, The fractions corresponding to the ribosome subunits were layered on a 7–52% sucrose gradient and the radioactivity was measured as described in Fig 1 The bottom of the gradient is on the left side of the abscissa.

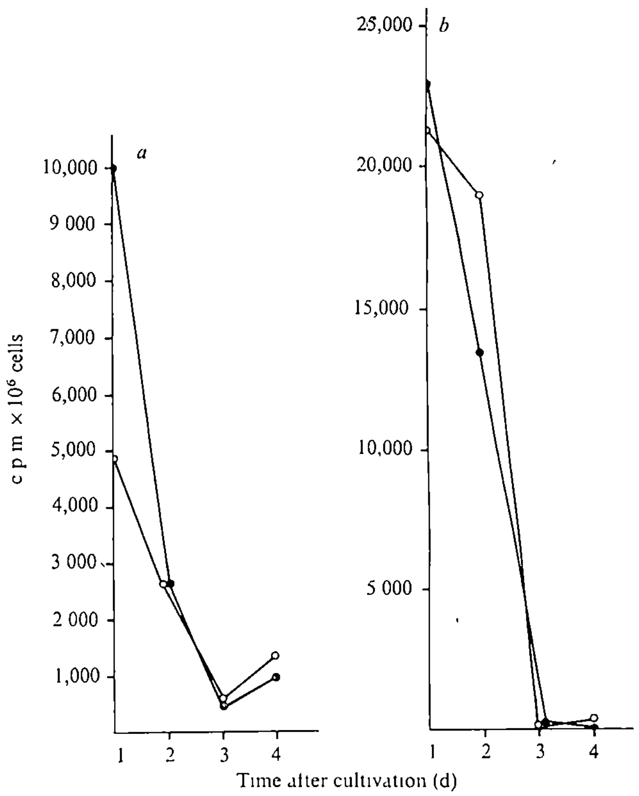

Fig 4 Incorporation of ^{14}C-labelled amino acids into *a*, acid insoluble and *b*, acid soluble fractions each day after subcultivation of human fibroblasts carried in medium with (●——●) and without (O——O) the hormone Cells were washed with phosphate-buffered saline (PBS) and then left for 15 min in PBS supplemented with 1 μCi ml^{-1} ^{14}C-labelled amino acids (Centre d'Energie Atomique, Saclay) (specific activity 0 1 Ci mmol^{-1}) The cells were scraped, washed three times in cold 10% TCA and the suspension was passed through millipore filters which were digested in Instagel The supernatants were pooled and 0 5 ml was added to 10 ml Instagel to measure the radioactivity in a liquid scintillator

The results confirm previous findings that cortisone[1] and hydrocortisone[2] prolong the lifespan of human fibroblasts *in vitro* It is interesting, however, that when hydrocortisone is withdrawn from cells which have grown beyond the lifespan of the controls, the cultures die within two passages It has been suggested[16] that cell senescence is due to an accumulation of errors at the level of protein synthesis and consequently an increased production of faulty molecules If this is the case hydrocortisone does not prevent the accumulation of errors since the cells die when the hormone is withdrawn Our data would be compatible with the error hypothesis if it is postulated that the hormone delays the deleterious effects of the presence of faulty molecules by an overall stimulation of protein synthesis, by stimulation of ribosome synthesis

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Rejection of skin grafts in the nude mouse

We recently reported that in (C57BL6/Rij × A/HeJ) F$_1$ (=B6AF1) mice, in which cellular immunity was temporarily suppressed by anti-lymphocyte serum, established B10 D2 skin allografts were hyperacutely rejected after the administration of specific alloantibody and rabbit complement[1] From this it was concluded that mouse skin allografts are sensitive to the cytotoxic action of alloantibody *in vivo* provided an effective species of complement is present at the same time

Nude mice do not reject allogeneic or xenogeneic skin grafts This phenomenon is usually explained by their inability to mount an effective cellular immune response[2,3] They, therefore, might serve as an ideal model for studying the effects of antibody on the fate of skin grafts We now report preliminary results of experiments in which acute rejection of skin allografts by antibody was induced in nude mice

B10 D2 (H-2^{d}) tail skin was grafted on to nude recipients by the procedure of Billingham and Medawar[4] The nude trait was bred on to a B10 LP (H-2^{b}) background to produce a congenic line, according to the method described by Snell[5] Mice of the second backcross generation were used In all experiments donor and recipient were of the same sex The fate of the grafts was followed by macroscopic inspection Untreated nude mice carried their grafts until death without any signs of rejection To induce acute rejection, nude mice in the experimental group received a single intravenous injection of 0 5 ml B6AF1 anti-B10 D2 serum (antiserum to H-2 31)[1] and two injections of 0 25 ml of rabbit complement, on day 8 after transplantation The antiserum was prepared by injecting B6AF1 recipient mice at weekly intervals with a suspension of B10 D2 lymphoid cells in complete Freund's adjuvant After five injections the animals developed ascites and could be tapped every 1 to 2 weeks The ascites fluid was pooled and tested with B10 D2 lymphoid cells by using the trypan blue dye exclusion method Two different pools were used, both having an antibody titre of 1 512 Fresh frozen sera from rabbits were used as the source of complement

Table 1 Effects of intravenous injection of B6AF1 anti-B10 D2 serum and rabbit complement on the survival of B10 D2 skin grafts in nude mice

Group no	No of recipients	Treatment: Alloanti-serum* to H-2 31	Treatment: Rabbit† complement	No of grafts showing rejection: Complete	No of grafts showing rejection: Patchy necrosis
1	6	+	+	4	2
2	5	+	–	0	0
3	3	+	+, inactivated	0	0
4	4	–	+	0	4
5	4	–	+, absorbed	2	2
6	4	–	+, inactivated	0	0

* 0 5 ml intravenously on day 8
† 0 25 ml intravenously on day 8 Second injection of 0 25 ml 8 h later

The results are presented in Table 1 All mice which received injections of antiserum and rabbit complement showed necrotic changes of their grafts within 2 d after the injection (group 1) After 4 d four grafts were completely destroyed, while the other grafts showed extensive necrosis leaving only 40–50% of the epithelium intact After the injection of antiserum alone no changes in the grafts were seen (group 2) If rabbit complement was inactivated by heating at 56° C for 45 min before its injection with antiserum, the grafts did not show signs of rejection (group 3) But in the control experiments where fresh frozen rabbit serum was given without the antiserum, grafts showed patchy necrosis after 24–48 h (group 4) This latter finding might be the result of the presence in rabbit serum of antibodies directed against mouse cells[6] This was unlikely, since the rabbit serum was not toxic to mouse cells *in vitro* Furthermore, it could still induce graft destruction after previous absorption with B10 D2 lymphoid cells (1 ml of packed cells to 5 ml of rabbit complement) for 10 min at 4° C (group 5) After its inactivation at 56° C for 45 min the rabbit serum did not cause destruction of the grafts (group 6) The deleterious effect of heat-labile factors in the rabbit serum on the skin grafts (groups 4 and 5) might be explained by the fact that the nude mice were able to form antibodies directed against B10 D2 antigens, which could induce acute destruction of the grafts after the addition of an efficient complement to the system We, therefore, tried to demonstrate the presence of antibody activity directed against B10 D2 lymphoid cells in the sera of nude mice that carried a B10 D2 skin graft The mice were bled on day 8 after transplantation, and the sera were tested in a trypan blue assay[1] Different dilutions of serum (0 1 ml) and of cell suspension (0 1 ml, 5×10^5 cells) were incubated with 0 1 ml of rabbit complement at 37° C for 30 min Figure 1 shows that there was definite cytotoxic activity in these sera C57BL6/Rij (H-2^{b}) lymphoid cells, serving as control cells, were not lysed by the serum Several mice were tested separately, and all showed comparable antibody activity

Three conclusions can be drawn from these results First, the experiments show that skin grafts are sensitive to the cytotoxic action of alloantibody *in vivo* in a model where cell-mediated immunity is virtually absent Second, it is clear that nude mice are capable of forming antibodies directed against transplantation antigens This is in accordance with other studies where these mice were shown to

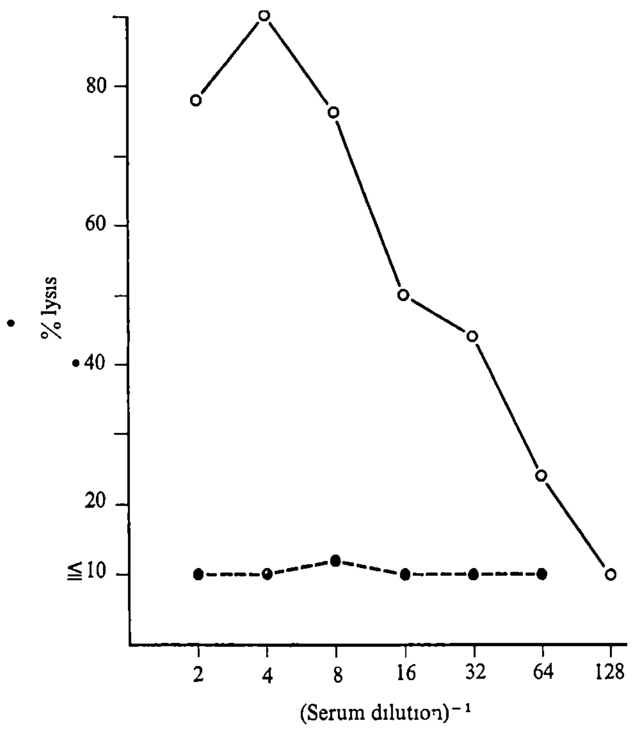

Fig. 1 Cytolysis of O, B10 D2 and ●, C57BL6/Rij lymphoid cells by nude serum and rabbit complement Mice were bled at day 8 after transplantation

form antibodies against thymus-dependent antigens, for example, sheep red blood cells[7,8] Third, the results of our experiments suggest that the antibodies, which are formed after skin grafting, are able to destroy these grafts if an efficient species of complement is added to the system The mouse native complement is very inefficient, at least in *in vitro* cytoxicity systems[9] This has been established not only in strains with a genetically determined deficiency of the C5 component of the complement system, but also in many other strains so far tested[10] It probably holds true for the species as a whole We found in our control experiments that fresh nude serum collected at day 8 after grafting, could only lyse B10 D2 cells after the addition of rabbit complement This suggests that also in this strain endogenous complement is relatively inefficient *in vitro* The indefinite survival of skin grafts in nude mice is probably not only caused by the insufficiency of the cellular immune response in these animals, but also by their lack of an efficient complement system

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Spontaneous sister chromatid exchanges detected by a BUdR-labelling method

THERE is still controversy over the nature of sister chromatid exchanges detectable by autoradiography Based on the relation between the number of exchanges and dose of tritium incorporated into DNA, Gibson and Prescott[1] have concluded that all exchanges are radiation-induced and that none occurs spontaneously But such an approach has an apparent technical drawback in that the declining of sensitivity of emulsion to tritium as well as latent image fading during long autoradiographic exposure provides a definite lower limit in the number of grains formed at a given tritium dose, and hampers greatly the detection of exchanges at very low dose of incorporated tritium Another

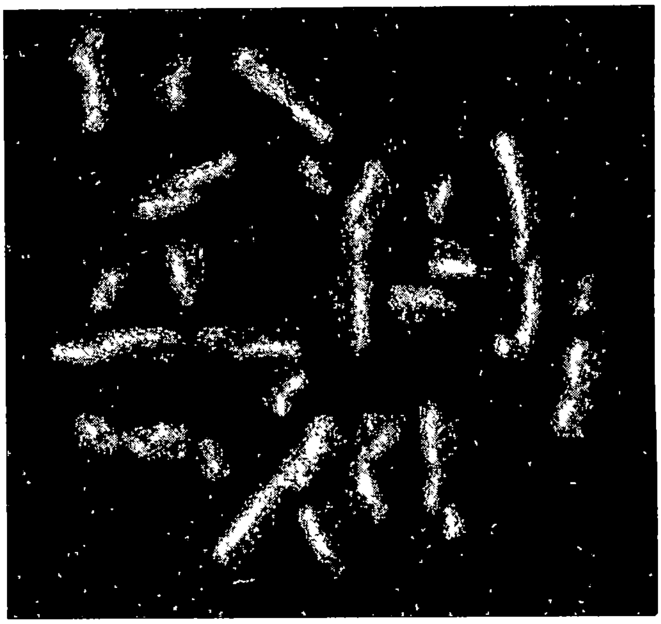

Fig 1 Fluorescence photomicrograph of metaphase chromosomes stained with acridine orange This cell had previously been labelled with 1 0 μg ml^{-1} BUdR for 26 h and fixed according to the air-drying method following 1 h Colcemid treatment × 1,600 The cell had an exceptionally large number of exchange sites (11 exchanges per cell) The brightly stained chromatid was probably composed of DNA with only one strand substituted with BUdR, the duly stained one being composed of DNA with both strands substituted with BUdR Demarcation was also possible between a chromatid containing a non-BUdR substituted DNA duplex and its sister chromatid composed of a duplex with only one strand substituted with BUdR These chromatids could be obtained by pulse-labelling cells with BUdR and then growing them for another cell cycle in the absence of BUdR before fixation Such labelling procedures were not employed in this study so as to avoid a risk of exposure of BUdR-labelled cells to visible light which would cause breaks in BU-substituted DNA, and hence, would affect the frequency of sister chromatid exchanges

difficulty may arise from the fact that the exchange frequency reaches a saturation level at a very low dose and within a very narrow dose range, so that it is difficult to determine whether a dose response curve rises linearly with or as the square of dose Extrapolation of the curve through the zero point can be misleading To detect sister chromatid exchanges, I have, following the original work of Latt[3], used a new method involving labelling of chromosomes with BUdR followed by acridine orange fluorescence staining, and have obtained results that suggest strongly the existence of spontaneous sister chromatid exchanges

A pseudodiploid Chinese hamster cell line, D-6, was grown in total darkness in culture medium[2] containing BUdR at concentrations ranging from 0 1 μg ml^{-1} to 40 μg ml^{-1} for two cell cycles and fixed for chromosome preparation Slides were stained with 0 125 mg ml^{-1} acridine orange in phosphate buffer, *p*H 6, for 5 min, washed for 15 min with and mounted in the same buffer Sister chromatids were demarcated clearly, one being stained brightly and the other dully (Fig 1) This method allowed unequivocal detection of exchanges of even very short segments of sister chromatids which would probably escape detection by the conventional autoradiographic method The mechanisms underlying this differential staining remains to be elucidated Fluorescence of acridine orange might also be affected by a quenching effect of BUdR as reported for 3325 Hochest fluorescence[3]

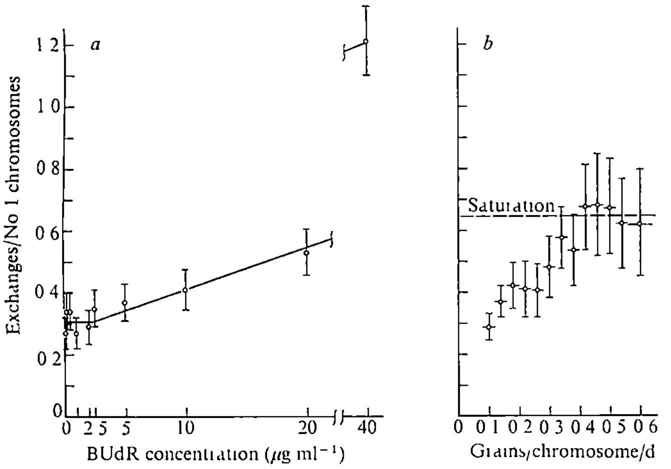

Fig 2 *a*, Sister chromatid exchanges scored in No 1 chromosomes of BUdR labelled cells Each point represents 100 chromosomes scored Range bars indicate s e m *b*, sister chromatid exchanges scored in No 1 chromasomes of cells which had been labelled with ^{3}H-thymidine (0 005 μCi ml^{-1} and 0 01 μCi ml^{-1}, specific activity 12 1 Ci mmol^{-1}) for 4 h and fixed in the second postlabelling mitosis Slides were coated with emulsion (Sakura NR-M2) and exposed for 132 d The number of grains as well as of exchange sites were scored in 755 no 1 chromosomes Range bars represent s e m and the horizontal line for each point indicates the range of grain number used to determine each point The broken line marked 'saturation' indicates the level of the maximum exchange frequency at high doses of incorporated tritium

In Fig 2*a* is plotted against BUdR concentrations the frequency of sister chromatid exchanges in No 1 chromosomes The frequency at 0 1 μg ml^{-1} was about 0 3 exchanges per chromosome and it remained constant up to 2 0 μg ml^{-1} With further increase in the concentration, the frequency started to rise and reached a level about four times of the constant level at 40 μg ml^{-1} Since BUdR labelling at concentrations lower than 0 1 μg ml^{-1} did not permit unequivocal demarcation of sister chromatids, it is uncertain whether the constant portion of the curve indicates a spontaneous exchange frequency or whether it would fall abruptly to zero at a lower concentration Results obtained with the conventional autoradiographic method are given in Fig 2*b* for comparison A dose-dependent increase in the exchange frequency was observed within the dose range 0 1 grains to 0 4 grains per chromosome per d confirming previous workers' results[1] The fate of the frequency curve at lower tritium doses is unknown, however a simple extension of the declining curve does not reach zero but meets the ordinate at a point between 0 2 and 0 3 exchanges per chromosome, which approximates to the value of the constant portion of the frequency curve obtained in BUdR-labelled cells (Fig 2*a*)

In Fig 3 the response to BUdR of sister chromatid exchanges and chromatid breaks are compared It is noticeable that frequencies of both events stayed at constant levels between 0 1 μg ml^{-1} and 1 0 μg ml^{-1}, and that the constant level of the frequency of breaks was exactly the same as that of non-BUdR treated controls These results suggest strongly that the constant portion of the frequency curve of sister chromatid exchanges might also represent a spontaneous exchange frequency Other possibilities, however, cannot yet be ruled out completely, for example (1) the genesis of sister chromatid exchanges might be completely different from that of chromatid breaks, so that the comparison of the two events in terms of their responses to BUdR might be meaningless, (2) there may be an upper limit for the BU substitution sites in DNA at low BUdR concentration[4], and such a limited level would be easily saturated, leaving the frequency of BUdR-induced sister chromatid exchanges constant

Since the exchange frequency observed between 0 1 μg ml^{-1} and 1 0 μg ml^{-1} (2 3 exchanges per cell) is the sum of exchanges formed during two successive cell cycles, the average frequency per cell generation is 1 15 If this frequency is ascribed to spontaneous exchanges, the rate may seem to be rather high considering that strand exchange

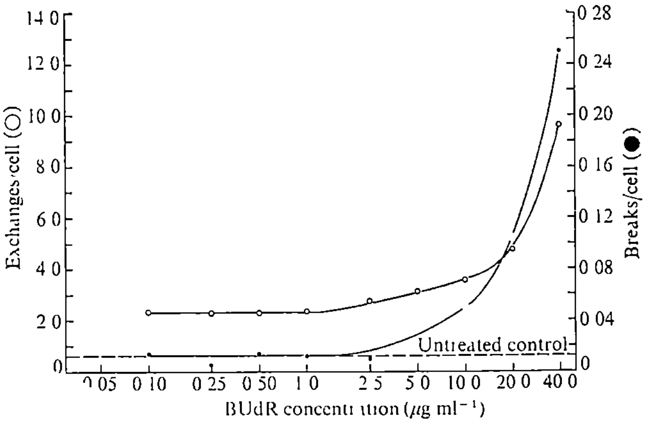

Fig 3 The number of sister chromatid exchanges per cell (○) is shown together with the frequency of breaks per cell (●) Each point on the curve for the exchange frequency was determined based on 50 acridine orange-stained cells Chromatid-type aberrations were scored in cells stained with Giemsa Each point on the frequency curve represents 200 cells scored, and is plotted in a 50-fold larger scale than that for sister chromatid exchanges Chromosome-type aberrations, both the 'derived-type' aberrations and those induced in G1, were omitted from the score Thus, the value presented in this figure is an underestimation for breaks per cell per generation Chromatid gaps were detected at almost the same frequency as that of chromatid deletions but they were omitted from this figure The broken line indicates the frequency of breaks observed in the non-BUdR treated control Whereas sister chromatid exchanges occurred randomly in every chromosomal complement at seemingly nonspecific sites, chromatid breaks as well as chromatid gaps were detected only in a few specific chromosomes such as No 1 and X The break points were mostly confined to constitutive heterochromatin regions

is an error-prone process[5]. But data showing that spontaneous DNA damage probably occurs to a significant extent in the normal conditions are accumulating. Lindahl and Nyberg[6] estimate that 2,000–10,000 purines would be lost from mammalian DNA in each cell generation. These apurinic sites seem to be repaired continuously by a maintenance system similar to the excision repair mechanism[7]. Spontaneous unscheduled DNA synthesis detectable in mammalian cells may be regarded as an outcome of such an error-correcting mechanism[8]. Although exact molecular processes leading to the formation of sister chromatid exchanges remain to be elucidated, they seem to be intimately related to mechanism of DNA repair[2]. It is uncertain yet whether sister chromatid exchanges are the outcome of a normal repair process or of errors in repair mechanisms. Whichever they may be, it is possible that one out of several thousand damaged sites in DNA would fail to restitute but provoke strand exchanges. In the same context, chromosomal breaks probably result from misrepair of DNA damage. A spontaneous frequency of breaks per cell per cell generation was about 0.012. This would mean that sister chromatid exchanges occur at a frequency about 100 times of that of chromatid breaks in the normal conditions. This ratio seems to be variable when both events are induced by exogenous agents (Fig. 3).

The observation that BUdR at high concentrations readily induced sister chromatid exchanges may be relevant to the findings that BU enhances the recombination frequency in λ phage and that a recombinational repair process is involved in BU-induced mutagenesis[9].

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Non-random loss of human markers from man-mouse somatic cell hybrids

THE preferential loss of human chromosomes from hybrids between human and mouse somatic cells[1] is being used in the assignment of human gene loci to chromosomes[2]. When the experimental design does not select for the gene product under study, the assignment often assumes that the loss of chromosomes is random. For example, hybrid clones derived from mouse cells and SV40-transformed human skin fibroblasts lose the SV40-induced T antigen only when all but a few human chromosomes have been eliminated[3]. If the pattern of chromosome loss is random, these results could indicate that SV40 DNA has integrated into the DNA of several chromosomes. They might be attributable, however, to the scheduled elimination of a single pertinent human chromosome relatively late in the evolution of each clone, a possibility supported by the finding of a preferential retention of C-7 in hybrids derived from SV40-transformed human cells and a concordance of this chromosome with the presence of SV40 and T antigen[4]. Moreover, if the loss of chromosomes were directed, joint segregation of two chromosomes might occur, making it difficult to decide which of the two specified the pertinent gene product being lost simultaneously.

We have studied the pattern of loss of human enzyme and chromosome markers from hybrid clones by means of single observations of many clones, as well as observation of the evolution of individual clones, which is a more sensitive measure of directed loss. Our findings suggest that in our system the elimination of human chromosomes is indeed not random.

As the murine parent, mouse fibroblasts (3T3) lacking thymidine kinase (EC 2.7.1.21, TK) were chosen because they can be eliminated by selective medium and because

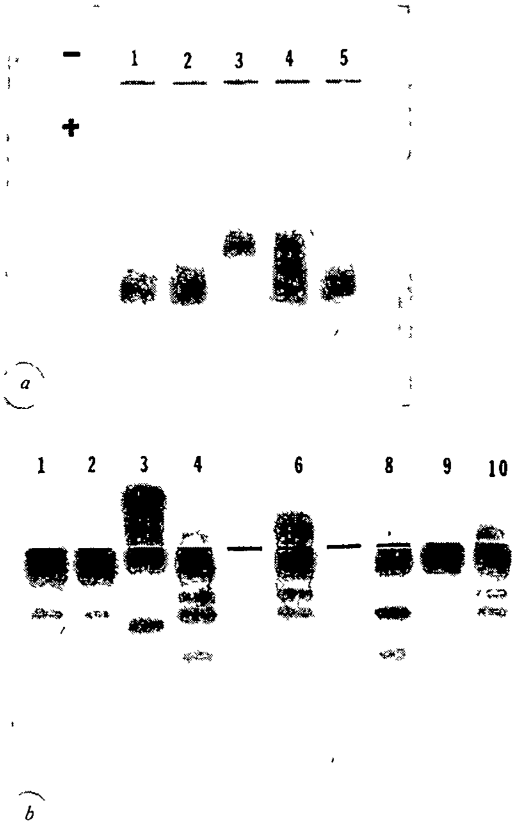

Fig. 1 Enzyme pattern of 3T3, human fibroblasts and hybrids. Electrophoresis of cell extracts[9] was performed on starch gels[10] which were stained for G6PD[11] or LDH[12]. *a*, G6PD zymogram: channel 1, 3T3, 2 and 5, hybrids without human G6PD, 3, normal human fibroblasts, 4, hybrid with human and mouse G6PD and heteropolymer. *b*, LDH zymogram, channels 1 and 2, hybrids without human LDH, 3, normal human fibroblasts, 4, 6, and 10, hybrids showing heteropolymers of human and mouse LDH A subunits and LDH B subunits presumably of human origin, 8, hybrid with LDH B subunits and no human LDH A subunits, 9, 3T3. The mouse fibroblasts have a single band of strong activity which migrates with mouse LDH 5 and therefore consists solely of LDH A subunits. The LDH B subunits of mouse and man share the same electrophoretic mobility, so that a band anodal to that of 3T3 indicates the presence of either mouse or human LDH B structural genes. Since the 3T3 parent has no B subunits, we have attributed any novel bands anodal to that of 3T3 to the presence of a human gene influencing this phenotype. Isozymes migrating cathodally to that of 3T3 were ascribed to the presence of the human LDH A subunit. Combinations of novel bands anodal and cathodal to that of 3T3 were interpreted as the simultaneous presence of human LDH subunits A and B

Table 1 Analysis of a hybrid clone maintained in HAT medium

Subculture no	LDH B	LDH A	G6PD
3	+	+	−
8	+	+	−
10	+	+	−
12	+	+	−
14	+	+	−
16	+	−	−

This table illustrates the method of analysis applied to each clone Cell extracts at various subcultures were assayed for human enzymes until the clone showed only one marker

the karyotype, containing almost exclusively acrocentric-telocentric chromosomes, cannot be confused with the human karyotype We used a clone with only a rare cell containing a biarmed chromosome To ensure a normal human parental karyotype, we used unstimulated leukocytes from a normal human male which were prepared[5] and washed twice with saline Fibroblasts (2×10^6) and leukocytes (2×10^7) were fused in suspension using β-propiolactone-inactivated Sendai virus[6] and plated in HAT selective medium[7] with amethopterin (10^{-7} M, Lederle) substituted for aminopterin

Forty HAT-resistant clones were isolated with cloning cylinders from 24 dishes between 13 and 33 d after fusion Multiple clones from a single dish were transferred individually 13 d after fusion As they were all about the same size, they presumably originated from different heterokaryons When these clones became confluent, half of each was plated into a dish containing HAT and the other half into a dish containing a non-selective medium, HT (HAT without amethopterin) Thus we compared the effect of amethopterin on the pattern of marker elimination At each subsequent subculture, one-fifth of the cells was used to continue the line and four-fifths were divided between two dishes, one for enzyme electrophoresis and one for chromosome preparations Lactate dehydrogenase subunit A (LDH A) and subunit (LDH B) (both EC 1 1 1 27) and glucose-6-phosphate dehydrogenase (G6PD, EC 1 1 1 49) were chosen as enzyme markers because mouse and human forms could be distinguished, because each is specified by a different chromosome[8] and because they could be analysed simultaneously

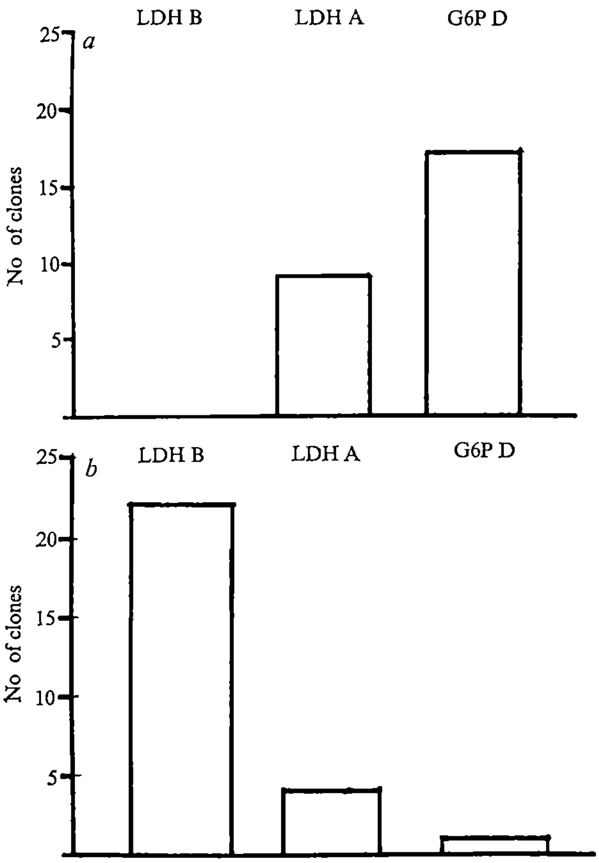

Fig. 2 Pattern of marker loss determined by serial observations of individual clones *a*, Number of clones losing each of the enzyme markers first, *b*, number of clones losing each of the enzyme markers last.

The characteristic G6PD patterns seen in our hybrids are shown in Fig 1*a* Only patterns with the G6PD heteropolymer were scored as positive for human G6PD Some of the LDH isozyme patterns observed are presented in Fig 1*b*

Thirty of the HAT-resistant colonies survived transfer and showed human isozymes and/or human marker chromosomes Each clone was analysed at various stages of its evolution until it had only one of the three enzyme markers (Table 1) Table 2 gives the frequency of the human enzymes in all hybrid clones analysed, including serial determinations of each during clonal evolution The distribution of the frequencies indicates that markers were not lost randomly

The predominance of LDH B reflects the timing of its elimination from individual hybrid clones (Fig 2*a* and *b*) Since amethopterin in the medium did not affect the incidence of the human LDH subunits and only slightly influenced the retention of human G6PD (Table 2), our observations from the clonal sublines maintained in the two media have been pooled The first enzyme lost was human G6PD in 17 of the 26 clones analysed No clone lost human LDH B first (Fig 2*a*) The last of the three human enzyme markers lost was LDH B in 22 out of 27 clones analysed (Fig 2*b*) The number of clones either losing G6PD first or retaining LDH B until last is significantly different from the numbers expected if the loss were random ($P<0$ 01) Thus a distinct pattern of enzyme elimination was observed, characterised by the relatively early loss of human G6PD and the retention of LDH B

Table 2 Frequency of human enzyme markers in hybrid clones

	Enzyme		
Medium	LDH B	LDH A	G6PD
HAT	88% (80)*	68% (81)	38% (69)
HT	86% (109)	62% (107)	27% (106)
Total	87% (189)	64% (188)	31% (175)

*Number of different clonal extracts analysed for the presence of the pertinent marker The extracts came from a total of 30 clones assayed at various times during their propagation and no duplicates are included

To test whether our analysis had been influenced by a differential sensitivity of the assay for the three enzymes, we performed reconstruction experiments with parent cell lines After starch gel electrophoresis, the distribution of LDH A and B subunits in extracts of human leukocytes is approximately equal To determine the relative sensitivity of the electrophoretic assay, we used human skin fibroblasts rather than leukocytes, as the former seemed more closely to simulate the intracellular environment in the hybrid Various proportions of fibroblasts were mixed with 3T3 cells and the extracts were subjected to electrophoresis In our gel system human fibroblasts have predominantly LDH A subunits Both LDH A and G6PD were equally detectable when as little as 5% of the mixture was human extract Since the LDH B was slightly less visible than the other two marker enzymes in these preparations, the observed pattern of enzyme loss cannot be explained on the basis of differential sensitivity of the assay

The early loss of G6PD is not surprising, since only one human X chromosome is present in the hybrid and since our data indicate that this chromosome provided no great selective advantage in HAT medium. Nevertheless, the preferential retention of human LDH B compared with LDH A cannot be explained on the basis of chromosome dose and is evidence of a directed pattern of chromosome loss

The hybrid clones were analysed for the presence of human chromosomes 1, 3, 6, 7, 9, 11, 12, and Y because their distinctive quinacrine mustard (QM) fluorescence patterns[13] facilitated discrimination from rearranged mouse chromosomes The 3T3 clone used as mouse parent contained an occasional submetacentric chromosome at the time of fusion and these did not increase significantly Moreover, no mouse chromosomes had fluorescent patterns that could be confused with the human chromosome markers The karyotype of the human parent was characterised by intensely fluorescent centromeres of chromosome 3 and the terminal long arm of the Y The 52 hybrid karyotypes analysed included 1 to 4 cells from each of 16 clones Most hybrid cells had two sets of 3T3 chromosomes

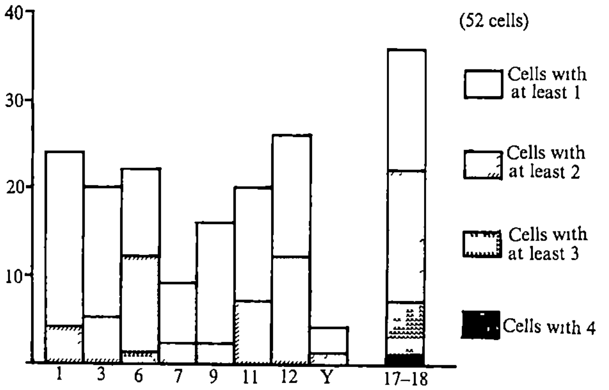

Fig 3 Frequency of human chromosome markers in hybrid cells

Because it is difficult to distinguish invariably chromosome E17 from E18, we have referred to a submetracentric E group chromosome as 17–18 However, we believe the chromosome is E17, since the thymidine kinase it specifies is essential to the survival of the hybrid cell

Except for chromosome 17, the incidence of human chromosome markers did not differ significantly in HAT and non-selective medium Therefore, we have combined observations of clonal sublines maintained in the two media for the analysis of the frequency of the various human chromosome markers (Fig 3) The least frequently observed chromosome was the Y, possibly because the parent line contained only one The most frequent was the chromosome specifying thymidine kinase[14] as a result of its selective retention by the culture conditions Moreover, we noted significant variation ($P<0\ 01$) in the frequency of the autosomes not obviously favoured or disfavoured by the experimental conditions Therefore, the distribution of chromosomes in the hybrid cells is not that expected if all chromosomes had an equal chance of inclusion in the hybrid genome In addition, the presence of human LDH subunits, together with the prevalence of chromosome C-11 and C-12 in these hybrids, supports the assignment of LDH A and LDH B to these two chromosomes[15,16]

Data from studies of other hybrids suggest to us further evidence of non-random human marker loss None of the nine clones derived by Santachiara *et al*[17] from fusion of 3T3 with human leukocytes of female origin had human G6PD, while four had human LDH A and seven had human LDH B Allderdice *et al*[18] studied the karyotype of man–mouse hybrid clones where selection favoured retention of the human X Of the chromosome markers used by us and Allderdice *et al*, C-12 was second in incidence only to the X, while C-11 was also frequently observed Further evidence of preferential inclusion of chromosomes specifying LDH in the hybrid is that a disproportionate number of autosomal linkage assignments made on the basis of hybrids involved the joint segregation of the pertinent marker with A or B subunits of LDH

The basis for the directed loss remains obscure, but may be related to the nature of the gene products specified by the chromosomes preferentially lost In this case one might predict that the pattern of elimination could vary with the parental cells and culture conditions used

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Isolation and *in vitro* translation of soybean leghaemoglobin mRNA

LEGHAEMOGLOBIN (Lb), a myoglobin-like protein, is found in large quantities in the root nodules which result from the symbiotic association between the bacterium *Rhizobium* and plants of the family Leguminosae The probable function of Lb is the regulation of intranodule oxygen tension[1] and its presence is correlated with rhizobial nitrogenase[2] The haem prosthetic group of Lb is of bacterial origin[3] and the plant contains the genetic determinants for the apoprotein[4,5] Soybean Lb consists of two major components of different electrophoretic mobility[6] The faster component (LbF) is composed of 139 amino acids, has a molecular weight of 15,600 and contains an amino terminal glycine residue, while the slower component (LbS) is com-

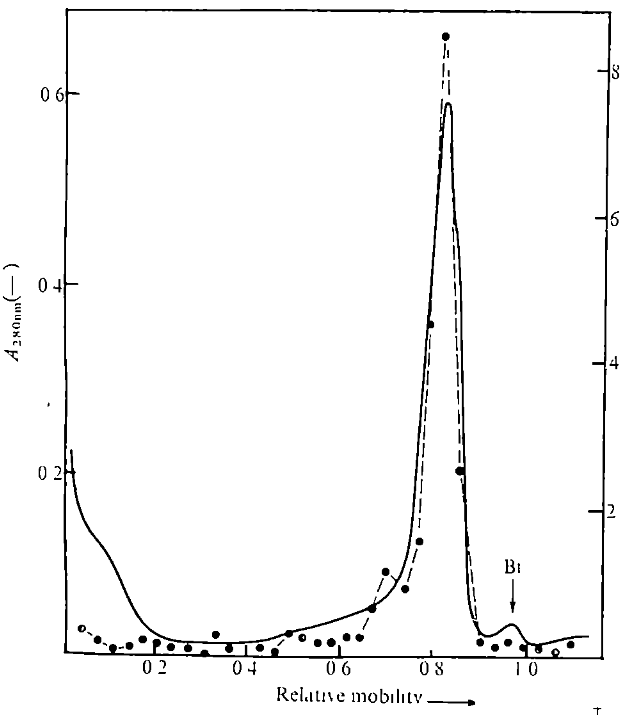

Fig. 1 SDS gel electrophoresis of the immunoprecipitated product from *in vitro* protein synthesis with nodule polysomes. Polysomes were isolated from nodules by homogenising the tissue in high ionic strength buffer (150 mM Tris-acetate, 20 mM KCl, 5 mM Mg acetate, 200 mM sucrose, 5 mM mercaptoethanol, 0.4% Nonidet P-40 detergent, *p*H 8.5). The polysomes were centrifuged through a 2 ml sucrose cushion (1.5 M sucrose, 50 mM Tris-acetate, 20mM KCl, 5 mM Mg acetate, *p*H 8.5), for 2 h at 105,000*g*, and resuspended in 10 mM Tris-HCl, 20 mM KCl, 1 mM Mg acetate and 20% glycerol. 4–8 A_{260} units of polysomes were incubated with the standard amino acid incorporation system in 400 μl consisting of 80 μl of postribosomal wheat supernatant, 45 mM KCl, 3.5 mM Mg acetate, 1 mM ATP, 25 μM GTP, 8 mM creatine phosphate, 16 μg creatine phosphate kinase, 25 mM Tris-acetate, *p*H 8.0, 2 mM dithiothreitol, 2 μCi 4,5-^{3}H-L-leucine (specific activity 64 Ci mmol^{-1}). After 60 min of incubation at 30° C carrier antigen was added (2 μg each of LbF and LbS) and ribosomes were removed by centrifugation. The postribosomal supernatant was reacted with anti-Lb serum for 60 min at 30° C. The immunoprecipitate was washed three times with phosphate-buffered saline, solubilised in SDS buffer and subjected to electrophoresis in 10% SDS gels[8]. Gels were cut into 2 mm slices and dissolved in 0.8 ml of 30% H_2O_2 at 55° C and counted in PCS (Amersham, Searle). The absorbance at 280 nm was determined on an identical gel run with Lb as a marker (50 μg each of LbS and LbF).

posed of 143 amino acids, has a molecular weight of 15,900 and contains an amino terminal valine residue. Because of these differences LbF and LbS are presumed to be different gene products[7]. Thus they are useful markers in studies on the transfer of plant genes involved in symbiosis to other plants.

In an attempt to elucidate the interaction between plant and bacteria in the expression of Lb genes we have isolated a poly(A)-containing 9S–12S mRNA from polysomes of soybean root nodules and translated it in a wheat embryo cell-free system. This mRNA can initiate protein synthesis on 80S wheat ribosomes and release completed protein chains which react with monospecific antibodies to Lb. Translation of Lb mRNA is not dependent on the presence of haem or any other factor from soybean root nodules.

Rhizobium japonicum strain 61A76 (supplied by Dr Joe Burton, Nitragin Co., Milwaukee) was grown in a liquid culture medium composed of (g l^{-1}) K_2HPO_4, 0.5, $MgSO_4 \cdot 7H_2O$, 0.2, NaCl, 0.1, yeast extract, 1.0, mannitol 10.0 and adjusted to *p*H 6.6. Bacteria were grown at 27° C with constant shaking and subcultured every 7 d. Soybean (*Glycine max*, var. Kanrich), was grown on Vermiculite in a controlled environment room under a 12 h photoperiod at 27° C and a night temperature of 21° C, and provided with a nitrogen-free nutrient solution[3]. Seeds were inoculated with a suspension of rhizobia in 5% sucrose before sowing and root nodules were collected 3–4 weeks later. The nodules were frozen immediately in liquid nitrogen and those 2–4 mm in diameter were used, except where mentioned otherwise.

Polysomes were prepared for translation experiments as outlined in the legend to Fig. 1. To test their capacity for protein synthesis, polysomes were incubated in a cell-free system containing postribosomal supernatant from wheat embryos[9–11]. Under these conditions polysomes primarily complete and release nascent protein chains (our unpublished data). The radioactive product from this system reacted with anti-Lb serum obtained from rabbits immunised with electrophoretically homogeneous Lb. Since the antiserum was monospecific and the radioactive antigen (after solubilisation from the antigen-antibody complex) migrated with

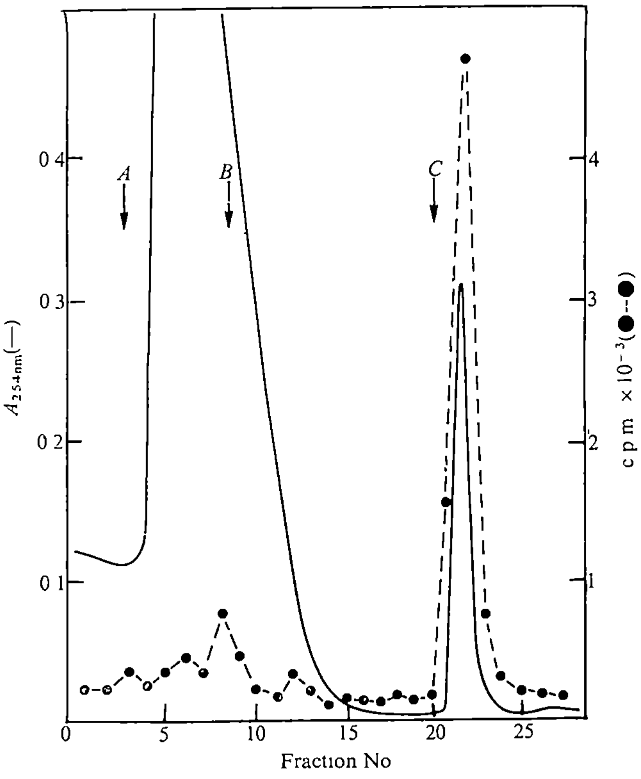

Fig. 2 Isolation of poly(A) containing mRNA by oligo(dT)-cellulose chromatography. Polysomes, prepared as for Fig. 1, were suspended in buffer (0.1 M Tris-acetate, 0.1 M NaCl, 2 mM Na EDTA, *p*H 9.0) and made 1% with respect to SDS at room temperature. Total polysomal RNA was extracted and chromatographed as described by Aviv and Leder[12]. The sample was applied at room temperature in SDS buffer (*A*). SDS was removed in the wash buffer (*B*). Poly(A) containing RNA was eluted with 0.01 M Tris-acetate, *p*H 7.4 (*C*). Absorbance at 254 nm was monitored with a Beckman DB spectrophotometer. An aliquot of each fraction was incubated with 5 nCi of ^{3}H-poly(U) in a final volume of 0.5 ml containing 0.2 M NaCl, 5 mM Mg acetate, 10 mM Tris-acetate, *p*H 7.4. Hybridisation was allowed to proceed for 15 min at 25° C, after which each fraction was treated with 20 μl of pancreatic RNase (20 μg ml^{-1}) for 30 min at 25° C. The reaction was terminated by adding cold 5% trichloroacetic acid (TCA). Carrier RNA was then added and the precipitates collected on Whatman GF/C filters, washed with cold 5% TCA, dried, and their radioactivity measured. No ^{3}H-poly(U) binding was observed with controls which consisted of equivalent, amounts of wheat ribosomes and/or wheat tRNA. The fractions which eluted with buffer (*C*) contained most of the poly(A) containing RNA, were pooled and made 2% with respect to potassium acetate, *p*H 5.5. They were then precipitated with 2.5 volumes of ethanol at −20° C

Table 1 Translation of root nodule RNA fractions and the immunoprecipitation of the products

Experiment	RNA	Total incorporation (c p m)	Anti-Lb serum precipitable radioactivity (c p m)	% of total radioactivity precipitated by anti-Lb serum
1	Total polysomal RNA	10,716	2,679	25
2	*RNA not containing poly (A)	237	—	—
	*Total poly (A)-containing RNA	40,320	11,290	28
3	†9S RNA	27,250	15,465	57
	†12S RNA	38,000	12,780	33

RNA fractions were translated in the standard amino acid incorporating system from wheat embryos in a total volume of 200 μl (experiment 2) or 400 μl (experiments 1 and 3) as described in Fig 1 After 60 min of incubation at 30° C an aliquot of the postribosomal supernatant was incubated with anti-Lb serum and processed as in Fig 1 Aliquots treated with normal rabbit serum precipitated 150–250 c p m in all cases *Prepared by oligo(dT)-cellulose chromatography (Fig 2)

†From sucrose gradients (Fig 4)

carrier Lb on sodium dodecyl sulphate (SDS) gels (Fig 1) we concluded that these polysomes contained mRNA which codes for soybean Lb

RNA was extracted from nodule polysomes and fractionated on a column of oligo (dT)-cellulose[12] The RNA which eluted from the column with low salt buffer was found, by hybridisation with ^{3}H-poly(U), to have most of the poly(A)-containing RNA (Fig 2)

The RNA fractions from the column were tested for ability to support protein synthesis in the wheat embryo cell-free system, which in addition to ribosomes contains all the factors necessary for protein synthesis but has very little endogenous mRNA activity[9–11] Only the poly(A)-containing RNA supported the incorporation of ^{3}H-leucine into released polypeptide chains (Table 1) Other RNA fractions were ineffective by themselves and were not inhibitory when added together with poly(A)-containing RNA Of the soluble radioactive products synthesised in the presence of the poly(A)-containing RNA, 25–30% was precipitated by anti-Lb serum The immunoprecipitated radioactivity migrated with carrier Lb on SDS gels (Fig 3), similar to the products made by total polysomes (Fig 1)

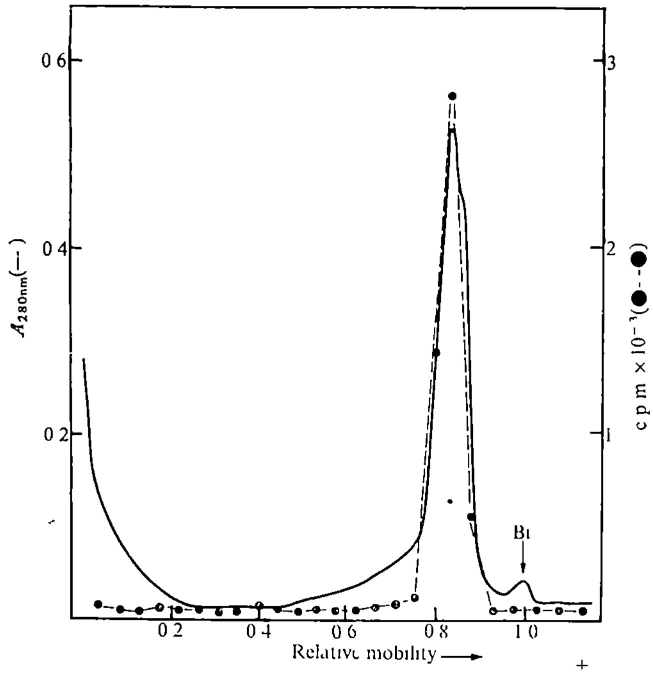

Fig. 3 SDS gel electrophoresis of the immunoprecipitated product from *in vitro* translation of leghaemoglobin mRNA Poly(A) containing RNA prepared from polysomes as in Fig 2 was translated in the standard wheat embyro amino acid incorporating system in a total volume of 400 μl (Fig 1) containing 4 A_{260} units of wheat embryo ribosomes After incubation, the postribosomal supernatant was reacted with anti-Lb serum and analysed in SDS gel as described in Fig 1.

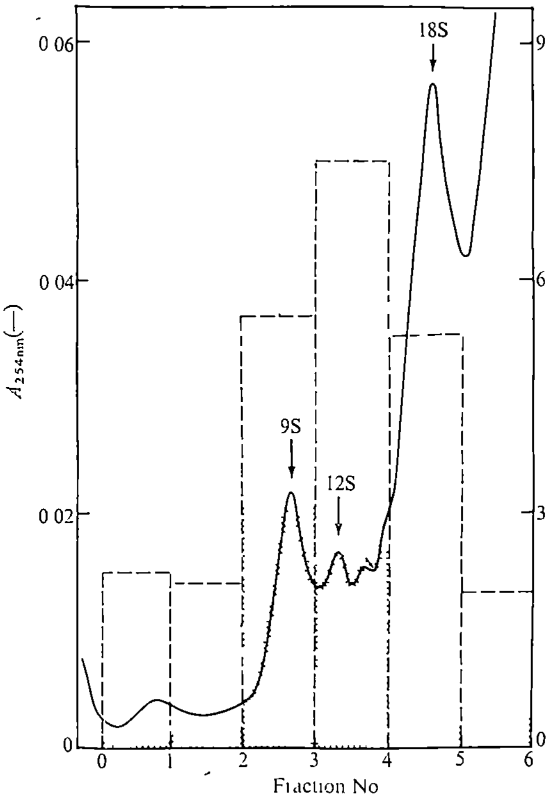

Fig 4 Sucrose gradient centrifugation of poly(A) containing RNA from soybean root nodules Poly(A) containing RNA was isolated (Fig 2), mixed with 100 μg of carrier RNA and layered on a 10 ml, 10–30% (w/v) sucrose gradient in 50mM Tris-acetate, 100 mM NaCl, 100 mM Na acetate, *p*H 8 5 Centrifugation was for 14 h at 39,000 r p m at 2° C in a Beckman SW 41 rotor Gradients were fractionated and A_{254} was monitored RNA from each fraction was precipitated at −20° C for 24 h with 2 5 volumes of ethanol The RNA was collected and dissolved in 40 μl of water Aliquots of these RNA fractions were translated in the standard amino acid incorporating system in a total volume of 200 μl containing 2 A_{260} units of wheat ribosomes (Fig 1) After incubation, an aliquot of each postribosomal supernatant was precipitated with hot 5% TCA and radioactivity was determined (total counts, open columns) while another aliquot was incubated with anti-Lb serum (shaded columns) for 60 min at 30° C The immunoprecipitate was washed twice with phosphate buffered saline and collected on Whatman GF/C filters Filters were washed with 5% TCA, dried and their radioactivity determined (anti Lb counts)

Analysis of the poly(A)-containing RNA by sucrose gradient centrifugation showed that it had mainly 9S and 12S components (Fig 4) Translation of the individual fractions from the gradient demonstrated that the 9S and 12S fractions were the most active in supporting protein synthesis in the wheat embryo system and the products from these fractions had the highest reactivity with anti-Lb serum (Fig 4 and Table 1) The S value of these RNA fractions was confirmed by electrophoresis in 2 8% acrylamide gels[13] The position of RNA in the gels was determined by ^{3}H-poly(U) hybridisation to fractions eluted from 2 mm gel slices

Since the two species of Lb in soybean root nodules (LbS and LbF) are equally reactive with Lb antisera and migrate together in SDS gels (Figs 1 and 3) it was not possible to determine whether the poly(A)-containing mRNA codes for one or both leghaemoglobins To answer this question the radioactive product was analysed with carrier LbS and LbF by disc electrophoresis[14]. Figure 5 shows that the bulk of the radioactivity accompanied the LbS whereas relatively little was associated with the LbF. The fact that the radioactivity was in Lb was confirmed by its reactivity with anti-Lb serum after elution of the corresponding sections from a duplicate gel. At present we have no explanation for the selective synthesis of LbS, since extracts of nodules of all ages contain more LbF than LbS (our unpublished data).

For its *in vitro* translation, Lb mRNA does not seem to require any specific factors which may arise in the nodule as a result of the symbiosis. Preliminary experiments suggest that the translation of Lb mRNA in the wheat embryo cell-free system is not stimulated by the addition of haem. Furthermore, it is unlikely that the system responded to endogenous haem, since the addition of human serum albumin, which can bind haem avidly[15], was without effect. It has also been shown that the translation of rabbit globin mRNA in the wheat embryo system does not require haem[16].

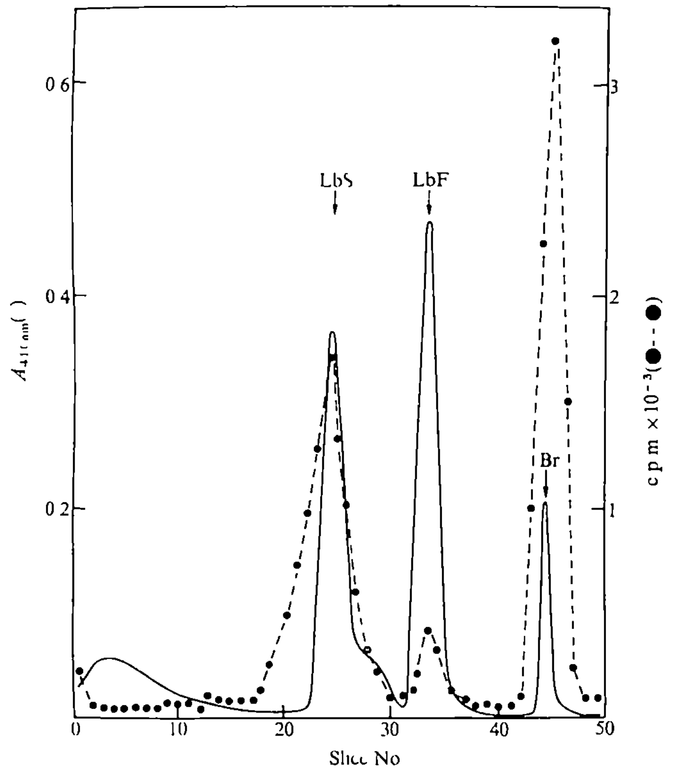

Fig. 5 Disc gel electrophoresis of the products made by poly(A)-containing RNA. The poly(A) containing RNA from Fig. 2 was translated in the standard wheat embyro system as described in Fig. 1 and the postribosomal supernatant containing released products was mixed with 10 μg each of LbS and LbF and analysed by electrophoresis (4 mA per tube) on 7.5% acrylamide gels[14]. After electrophoresis, gels were scanned at 410 nm in a Gilford spectrophotometer and their associated radioactivity was measured by fractionating the gels into 1 mm slices as described in Fig. 1. Br, bromophenol blue marker.

Although several eukaryotic mRNAs have been isolated and translated in various cell-free systems, this is the first report of the isolation and *in vitro* translation of a plant mRNA. Our experiments demonstrate that, like animal and viral mRNA, plant mRNA can be translated in a cell-free system derived from an unrelated species. Although isolated from a prokaryote-eukaryote symbiosis, Lb mRNA seems to be of plant origin since, like other eukaryotic messengers it contains poly(A) and is associated with 80S ribosomes[17–20]. These results are consistent with other evidence that the plant contains the genes for Lb[4,5]. Hybridisation of Lb mRNA with DNA from uninfected soybean plants should provide definitive proof of the location of the Lb gene.

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Differential staining of human and mouse chromosomes in interspecific cell hybrids

VIRUS-MEDIATED fusion of somatic cells to produce interspecific cell hybrids has proved to be a most valuable technique for biological research[1]. Such crosses often undergo an initial fairly random loss of many chromosomes of one parent before karyotypic stability is reached. This property can be used to generate clones of cells each containing a small selection of human chromosomes in a background genotype of some other species. Comparisons of such cell lines provide a form of segregation analysis which can substitute for more traditional genetic methods, particularly in studies on man where classical genetic methodology is difficult to apply.

Among the many interesting applications of this technique, a particularly prolific field has been the mapping of the human genome[2]. By studying the cosegregation of human gene products in a number of hybrid clones, linkage of the gene loci determining those products can be inferred

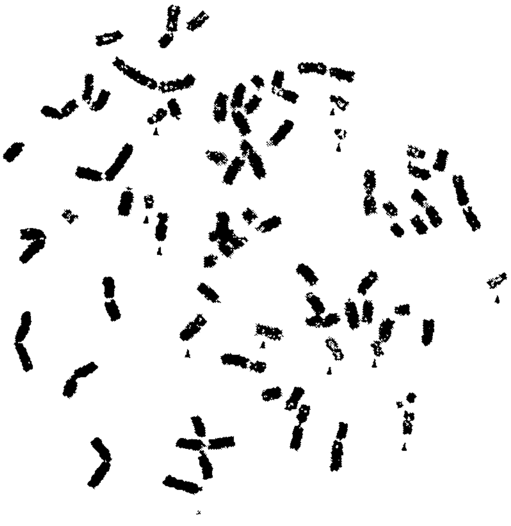

Fig 1 Metaphase of interspecific hybrid between mouse cell line (IR) and human lymphocytes Human chromosomes (arrowed) are pale blue, some having red paracentromeric heterochromatin Mouse chromosomes have pale blue centromeric heterochromatin and red euchromatic arms

If the karyotypes of the cell lines are established, gene loci can be assigned to particular chromosomes

The availability of well characterised cell lines and extensive genetic knowledge of the species, make mouse cells particularly suitable as 'carriers' in such studies A major disadvantage, however, is the difficulty of characterising the karyotypes of mouse–human cell hybrids Human acrocentrics, and abnormal chromosomes, are difficult to pick out of the complex and variable mouse background We have been investigating means of differentiating human from mouse chromosomes, to allow a fairly rapid screening of cell lines for the number and morphology of human chromosomes carried With this knowledge, formal identification of the individual chromosomes concerned using various chromosome banding techniques is greatly simplified We report here that mouse chromosomes, and the chromosomes of permanent mouse cell lines, show an almost exact reversal of the Giemsa-11 staining pattern found for human chromosomes, and that these staining characteristics are retained in mouse–human hybrid cells

The Giemsa-11 staining technique[3,4] highlights specific regions of constitutive heterochromatin in the human karyotype In optimal preparations, the chromosomes are a bluish colour, with red differentiation of paracentromeric regions of chromosomes 1, 4, 5, 7, 9, 10, 13, 14, 15, 20, 21 and 22 Parts of the Y chromosome are also stained Mouse chromosomes stain a dark magenta, with pale blue centromeres In hybrids, those human chromosomes which contain G-11 positive material are most readily identified, being pale blue with red paracentromeric regions (Fig 1) Even those human chromosomes such as the X, which have no G-11 positive material, can be differentiated from the surrounding mouse chromosomes by their lighter colour, and by the absence of the markedly paler centromere regions characteristic of mouse chromosomes

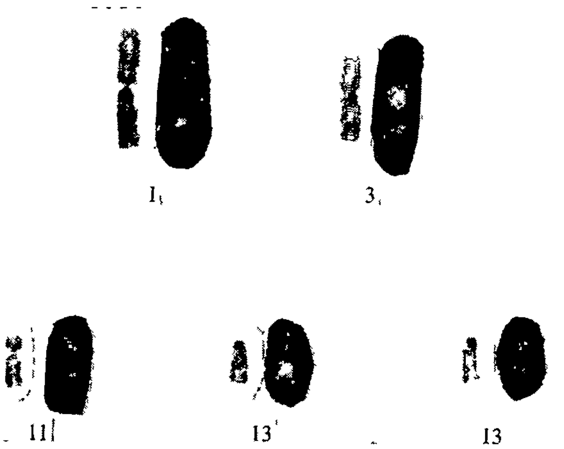

Fig. 2 Human chromosomes from a single mouse–human hybrid metaphase, identified by G-11 staining (left) and restained with quinacrine (right)

The staining procedure is similar to that used on diploid human cells Colcemid-arrested metaphases are treated for 15–20 min in 0 075 M KCl, fixed in 3 1 methanol acetic acid, and air dried Flame dried slides can also be used Slides are left for about 1 week at room temperature, or for 2 d in a dry oven at 60° C, before staining The staining solution is a 1/50 aqueous dilution of Giemsa (Harleco), adjusted to pH 11 0 with NaOH Optimal staining is usually achieved in 10–20 min Slides are then rinsed in pH 6 8 phosphate buffer and allowed to dry Unmounted slides can be examined and photographed with oil immersion objectives After removing the oil with xylene, they can be destained in two changes of acetic methanol and satisfactorily restained with quinacrine or G-banded using trypsin[5]

As with all chromosome banding techniques, the quality of staining is somewhat erratic But altering the staining time, and the pH and dye concentration of the staining solution, we have achieved success in 14 out of 15 hybrid clones studied The identity of the chromosomes concerned has been established by re-examining previously photographed metaphases under quinacrine fluorescence, (Fig 2), as well as by formally karyotyping Giemsa banded cells of the same clones One clone, known on biochemical evidence to contain only part of a human X chromosome, was found to have a chromosome which stained in part as a mouse chromosome and in part as a human chromosome (Fig 3) The pale staining of human euchromatin cannot be distinguished from mouse constitutive heterochromatin and although this chromosome has not been seen in either the parent mouse line or any of the 11 other hybrid clones derived from it, its characterisation as a mouse–human translocation cannot yet be regarded as final

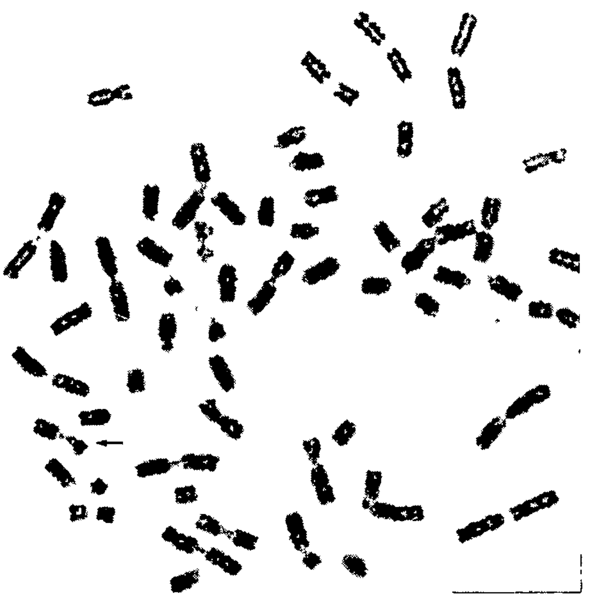

Fig 3 Partial metaphase of mouse–human hybrid (clone 2W1-R70) showing possible interspecific translocation chromosome (arrowed)

The physicochemical basis of the G-11 staining reaction remains obscure On human chromosomes, it was previously noted[3] that staining in alkaline Giemsa rapidly leads to an overall pale blue coloration suggestive of methylene blue, with the subsequent development of specific red staining at particular chromosomal sites This accords with the work of Summer and Evans[6] on G-banding, which led them to propose that the magenta colour of chromosome bands stained with Giemsa was due to the *in situ* formation of a molecular complex of methylene blue and eosin Giemsa-banding can also be produced by simple manipulation of the *p*H of the staining solution[7], suggesting that as the alkalinity of the stain is raised, an increasing amount of human chromatin loses the capacity to form such magenta dye complexes

The ability to form red dye complexes at high *p*H is clearly not a particular property of constitutive heterochromatin or of highly repetitious DNA sequences, since mouse heterochromatin exhibits the same colour changes as human euchromatin even when the chromosomes are in the same cell It seems likely that the change in staining is not due to a direct influence of *p*H on the process of dye binding, but is mediated by an alteration in the structure of particular regions of chromatin This could be as simple as a disruption of the compact coiling of the chromatin fibrils, due to an effect of *p*H on associated structural proteins, but the real nature of the process is still completely unknown

It is, however, clear that the G-11 technique can serve as a most useful method for the primary analysis of the chromosomes of human–mouse cell hybrids Coupled with re-examination of photographed metaphases under quinacrine fluorescence, it has provided us with an acceptable technique for the identification of human chromosomes against a mouse chromosome background Whether it will prove useful for other combinations of species has not yet been determined

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T lymphoblastoid cell lines from Marek's disease lymphomas

MAREK'S disease (MD) is a contagious lymphoproliferative condition of chickens caused by a herpesvirus It has the distinction of being the first commonly occurring tumour to be controlled by vaccination In its complex pathology and aetiology the disease bears remarkable similarities to Burkitt's lymphoma (BL), but the potential of MD as a model of human lymphoproliferative disease and of herpesvirus-induced neoplasia has not been fully realised One obstacle has been the lack of any established cell lines[1] This contrasts with studies on BL, where, in the absence of suitable experimental animals, attention has been focused on the growth of tumour cells *in vitro*[2] A continuous cell culture from a lymphoma of MD has recently, however, been reported[3] We now report the establishment of two cell lines from MD lymphomas and their characterisation as T cells

The approach used was that found to be successful in the long term culture of lymphocytes from BL biopsies[4] Ovarian lymphomas were collected from experimentally infected birds at about 5 weeks of age Single cell suspensions were prepared either by gentle teasing of the tumours or by agitation in 1% trypsin The suspensions were adjusted to 5×10^6 cells ml^{-1} and cultures of 5 ml volume were incubated in screw cap glass universal bottles at either 37° C or at 40° C Cultures of lymphocytes separated from heparinised blood samples were initiated in a similar way The medium used was RPMI 1640 supplemented with 20% foetal calf serum (FCS), and 10% tryptose phosphate broth All cultures were set up in replicates according to the number of cells available

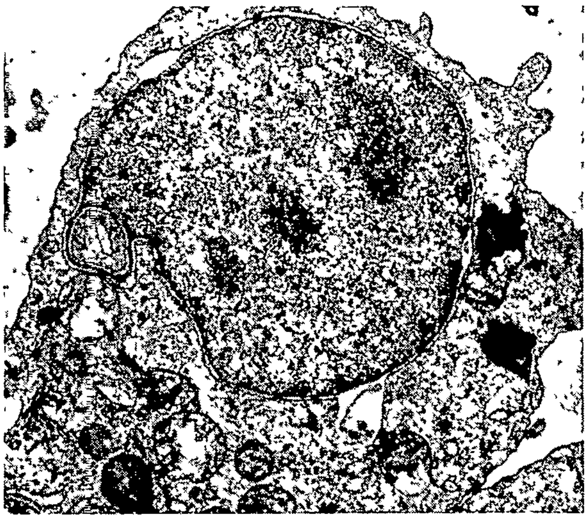

Fig 1 Typical lymphoblast of HPRS Line 1 The rounded nucleus has a projection of the nuclear envelope The cytoplasm contains many ribosomes, several mitochondria and osmiophilic bodies, but sparse endoplasmic reticulum (× 11,250)

Cultures were refed by replacing half of the supernatant medium, taking care not to disturb the cells, with fresh medium every 2 d for the first week of culture, and thereafter weekly

Several cultures gave rise to a profuse growth of adherent fibroblast-like cells Cultures were examined regularly for evidence of the growth of free floating cells One culture derived from an ovarian tumour was found to be growing in this manner 31 d after initiation This culture has now been growing for 29 weeks and is designated 'HPRS Line 1'. A second culture (HPRS Line 2), also from a lymphoma, was found to be multiplying 92 d after initiation This culture has now been growing for 13 weeks Cultures were divided into two every 7 or 10 d according to their appearance

In the growth of both cultures a high proportion of dead cells has been consistently observed Medium supplemented with 30% FCS or with 15% FCS and 15% chick serum supported growth, but did not correct this problem The cells were easily damaged by handling Growth was better at 40° C than at 37° C

The cells were never attached to glass They appeared singly or in pairs but not in clumps Most cells were round but a few had projections making them elongated The cells ranged in size, 'when measured in the living state, from 5 µm to 12 µm with a mean of 8 µm In smears the cells had large nuclei and a rim of intensely basophilic cytoplasm containing vacuoles Some large cells contained two nuclei Nucleoli were not seen

Ultrastructurally HPRS Line 1 cells were similar to cells seen in MD lymphomas (Fig 1) The nucleus was round or oval and sometimes slightly indented A nucleolus was occasionally seen and about 3% of the cells showed projections of the nuclear envelope (Fig 1) The cytoplasm contained many ribosomes, some of which were in the form of polyribosomes Several mitochondria were present, but the endoplasmic reticulum, which was often rough surfaced, was sparse A Golgi apparatus was seen in some cells Centrioles were fairly frequent and were often accompanied by spindle tubules Small, often irregularly shaped osmiophilic bodies, probably of lipid were normally present in the cytoplasm Occasional vacuoles were seen, and a small number of cells had parallel arrays of annulate lamellae in the cytoplasm Cells containing immature herpesvirus particles were seen rarely

HPRS Line 1 cells acted as plaque-forming infectious units to chick kidney cell monolayers Estimates of infectivity, each on several different subcultures, were made at four different times These gave values of 80, 1,048, 233, 1,278 plaque-forming units per million viable cells The cell lines do not produce cell free virus

Two cultures capable of long term growth have arisen from a total of 120 cultures, derived from 49 tumours and 11 blood samples A further 32 lymphocyte preparations from normal blood failed to grow in long term culture This contrasts with the relative ease with which lymphoblastoid lines may be established from human blood and BL biopsies, and suggests that the method described is not optimal, though it may be a reflection of the notorious difficulty of establishing avian cell lines[1]

Suspensions of living HPRS Line 1 cells were examined for membrane antigens using the indirect fluorescent antibody technique[5] The specific antisera were raised in chickens against HPRS 16 infected chick kidney cells (anti-MDV), and in rabbits against suspensions of MD lymphoma cells (anti-tumour), normal thymus cells (anti-T) and normal bursa cells (anti-B) All antisera had been extensively cross absorbed The results are shown in Table 1 The anti-B and anti-T sera were specific as assessed by the staining of viable thymus or bursa lymphocytes These sera stained B and T cells respectively in the peripheral blood The anti-MDV serum was specific for virus plaques in chick kidney cell cultures The high proportion of lymphoma cells and HPRS Line 1 cells staining with the anti-tumour antiserum suggests the presence of a tumour-specific antigen, though the staining of a small number of normal lymphocytes makes the specificity of the reaction questionable The results indicate that such a tumour-specific antigen is not identical to the known virus-associated antigens Line 1 cells stained for T-surface determinants, showing smooth membrane staining with capping, typical of T cells Line 2 cells have been examined for membrane fluorescence on one occasion 100% stained for T cell determinants, and 0% for B-cell determinants The identification of the cell lines as T cells is supported by the ultrastructural studies which showed the presence of abundant ribosomes and sparse endoplasmic reticulum

Table 1 Percentage of cells showing specific membrane fluorescence*

Antiserum	HPRS Line 1	Thymus suspension	Bursa suspension	Blood lymphocyte	MD lymphoma	MD virus plaques in CKC
Anti-thymus	99 5±0 3	93 9±2 3	4 0±0 4	73 5±2 8	76 6±3 9	—
Anti-bursa	1 1±0 4	4 3±0 3	94 0±0 9	21 8±3 3	20 4±2 4	—
Anti-tumour	96 3±1 2	0	0	1 3±0 2	35 1±9 6	—
Anti-MDV	1 0±1 0	0	0	0	2 8±1 8	+++

* Mean of five determinations ± Standard error

The majority of cells in Marek's disease lymphomas are of thymus origin[5,6] The role of these cells in the histopathogenesis of the disease is not clear The lymphoid system may be involved in the production of tumours by the malignant transformation of lymphoid target cells (intrinsic mechanism), or by the participation of lymphocytes in an excessive proliferative response to new viral antigen (extrinsic mechanism)[7] Since MD usually has a multifocal origin, an extrinsic mechanism is obviously a possibility There is, however, a poor correlation between the presence of viral antigen and the site of tumour development[8] Morever, BL is also frequently multifocal and yet is a uniclonal disease, this implies that the successful development of a neoplastic clone is a relatively rare event, and may depend upon an infrequent accident of interaction between virus and cells of an appropriate transformation type[1] Tumour-specific antigens have been reported[9] in MD the results of our fluorescent antibody studies suggest that the cell lines carry membrane tumour-specific antigens The presence of MD virus in the cells parallels the role of EB virus in the proliferation of human lymphocytes *in vitro* We suggest that malignant transformation of thymus-dependent lymphocytes is necessary for the development of lymphomas in Marek's disease

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reviews

Jacob as historian and biologist

The Logic of Living Systems By Francois Jacob Translated by Betty E Spillmann Pp viii+348 (Allen Lane London, 1974) £4

THIS book is a history of knowledge and ideas on reproduction and heredity It is intended by its author to be not merely a narrative account but a history which is in some sense explanatory, a history which shows how this knowledge was generated and how it changed

Jacob outlines his historiographic principles in the introductory "programme" where he rejects "reverse history"—which simply studies the succession of ideas resulting in our present views—as inadequate Rather, Jacob argues, a history of scientific knowledge and ideas must seek "to discover how objects become accessible to investigation", an "analysis of the nature of the object is required and of the attitudes of the investigators, their methods of observation and the obstacles raised by their cultural background" Using this approach, Jacob maintains, one no longer observes a linear sequence of ideas—"the confident march of progress"—each derived from its predecessors by a sort of evolutionary transformation, but instead a series of more or less self-contained and discontinuous "domains which thought tries to explore, where it seeks to establish order and attempts to construct a world of abstract relationships in harmony not only with observation and techniques but also with current practices, values and interpretations" For an object to be accessible to investigation a theory able to accommodate it must exist and "each period is characterised by a range of possibilities defined not only by the current theories and beliefs but also by the very nature of the object" This range of possibilities is the "domain", the territory within which reason can operate, and it is from these possibilities that theoretical "solutions" to problems are chosen When new theories are accepted they reorganise "the domain of the possible", modify ways of looking at things and bring to light new relationships and objects Historiographic principles of this sort, Jacob maintains, will result in a history which is concerned with "specifying the various stages of knowledge, defining the transformations and revealing the conditions which enable objects and interpretations to enter the field of the possible"

Jacob clearly has given much thought to the problems of writing history and the conceptual outline in his programme will be familiar to those who have some knowledge of that heterogeneous body of thought loosely labelled 'structuralism' His specific historiographic principles immediately bring to mind the work of Michel Foucault (and, more remotely, T S Kuhn) and it is not difficult to see the influence of Foucault's ideas on this book, even though Jacob makes no reference to him, or indeed to any contemporary historian

Jacob's careful statement of his historiographic principles and their nature led me to expect a work that would be comparable to the work of Foucault and to that of those other French students of cultural phenomena who are working within a broadly similar framework I expected a history of biology radically different from those produced within a more conventional, empirically oriented, historiographic framework, one full of new and provocative insights and interpretations I confess that I was disappointed This is a book of some distinction, certainly, it contains much of great interest, it is thought provoking, it is written in a style which is lucid and elegant and it deserves to be widely read Yet, it seems to me to fall considerably short of its stated goal, for Jacob has, in fact, produced a book which is almost a reverse history, and one that is relatively conventional, even old fashioned in some respects, especially as regards its rigorous internalism

It is not easy in a short review to do more than suggest why I found the book disappointing It is a complex work which resists easy generalisations and in what follows I am conscious that I have not done justice to it and that it requires and deserves a more detailed analysis

The book's shortcomings arise, I suspect, because of a tension or conflict between Jacob the biologist and Jacob the historian The first Jacob has made significant contributions to present knowledge and obviously has a certain commitment to it, he is concerned with the diachronic and with progress The second Jacob, in his role as historian, is concerned to do justice to his predecessors, he is interested in the synchronic but wary, perhaps, of the danger of relativism I suspect that the first Jacob had the upper hand in the writing of this book

In his discussion of the development of evolution theories, he remarks that "the role that is attributed to the past is necessarily conditioned by the way in which the present is viewed and interpreted" This remark could be applied to his own book, for in the last chapter he presents an interpretation of living systems as a hierarchical arrangement of organisations, one "enclosed" within the other like a nest of "Russian dolls", this structure provides the basis for Jacob's history which consists of a series of "stages of knowledge" in which each Russian doll is discovered Beginning in the seventeenth century with a domain of thought which is concerned with "visible structure"—the arrangements of matter, he passes to a second domain of "organisation" which occurs at the end of the eighteenth century and is concerned with the invisible unity of the organism, a unity which is later dissolved into the properties and interactions of cells, at the beginning of the twentieth century is the third domain in which chromosomes and genes are possibilities and, finally, our domain, that of the nucleic acid molecule Interposed between the discussions of the second and third stages is a chapter on "Time" in which ideas on the genesis and evolution of living things are considered

The series of historical stages which Jacob has constructed on the basis of his picture of the structure of living systems is not simply a sequence, it is a progress towards something hidden and more fundamental, it represents a journey to the metaphorical centre of the organism, a centre which for Jacob is also metaphysical, the source of order and change the genetic programme

This basic conception of progress often seems to undermine Jacob's theoretical principles He has a tendency on occasion to write with hindsight and to consider and pass judgement on past knowledge as simply a precursor of future, and therefore more fundamental, knowledge, rather than as a system possessing its own coherence Consequently, there are places where past thought is very simplified or even distorted This progressive picture also results in the omission of thought which does not fit into the pattern, the most notable example being twentieth century developmental biology

Jacob's rigorously internalist approach also seems to be in conflict with his historiographic principles and results in some disappointingly conventional interpretations Evolutionary thought, for example, is described in the programme as a product of "mid-nineteenth century thought as a whole", yet it is actually discussed in a very conventional fashion as an internal problem in biology, centring on the issue of the contingency of the living world, with a reference to similar ideas in contemporary statistical mechanics This, in my view, is neither an adequate nor a particularly illuminating account of a transformation in thought involving ideas on God, man, nature and society and their interrelations

On the other hand, problems also arise when Jacob applies his historiographic principles The notion of relatively discontinuous domains of thought results in an historical picture in which the real continuities which exist are often obscured, and one which is often too schematic His chapter on the "Visible Structure", which is concerned with seventeenth century thought, suffers as a consequence of this, even though parts of it are fascinating His belief that "mechanism, despite its limitations, represented the only attitude compatible with knowledge of that time" and that other metaphysical principles were not scientifically productive but were merely of "ethical" or "philosophical" significance, seems to me to do violence to the richness and complexity of seventeenth century thought and results in misleading discussions of, for example, the discovery of microorganisms and the circulation of the blood In these examples it seems that, although Jacob recognises that "objects" can form the subject of discourse in more than one domain, he believes that they are only accessible to investigation which is scientific and productive within a unique domain which is in "harmony" with their "nature" This presupposes that the "nature" of an "object" is completely independent of the theoretical framework in which that object is considered, a debatable assumption

The final chapter, "The Integron", is clearly written by Jacob the biologist, and contains a discussion of some current views of the organisation of living systems It is surprisingly uncritical and tends to confirm my suspicion that Jacob the biologist was the dominant personality in the writing of the whole book, for reverse or Whig history is, by and large, intended to justify the present state of affairs and to encourage us to accept it uncritically On the other hand, the sort of history in which Jacob the historian is interested, the sort which asks the question "How is it possible for anyone to believe that?", has the goal of making us ask the same question with respect to our own beliefs, and therefore, of freeing us from the spectacles of habit and from the tyranny of our own artefacts Jacob seems to be aware of this but evades coming to grips with it In the final paragraph of the book he writes "There are perhaps other possible coherences in descriptions But science is enclosed in its own explanatory system and cannot escape from it" This is to raise the possibility of freedom, and therefore the necessity of choice, and then immediately to reject it by picturing science as an autonomous and alien thing, the "Russian doll" as a Petrushka, an artefact with a life of its own It is to evade the fact that we are responsible for all our artefacts, including our "beliefs" and our "knowledge"

GERALD WEBSTER

Stimulating immunity

Chemical and Biological Basis of Adjuvants By P Jollés and A Paraf Pp viii+153 (Molecular Biology, Biochemistry and Biophysics, 13) (Springer-Verlag Berlin and New York, 1973) £7 90

AT best the subject of immunological adjuvants consisted of a potpourri of related phenomena the publication of this monograph has to some extent improved the situation

The authors have striven hard to obtain order and consistency which they achieved with respect to the chemical basis of adjuvants, although it has been done by introducing a noticeable degree of repetition I found the earlier chapters especially informative with regard to the adjuvant materials of mycobacterial origin In the area of cellular immunology, Jollés and Paraf have not achieved such clarity and there is no coherent discussion of immunopotentiation by substances acting on populations of differentiating cells

I found the last chapter on the practical uses of adjuvants very incomplete and nothing like as scholarly as the earlier chapters It would have been useful to have had a more critical and detailed discussion of recent French work where certain bacterial vaccines (*C parvum*, BCG) have been used in the treatment of leukaemias Direct toxicity is an important consideration and is adequately discussed, however only brief mention is made of the danger of adjuvants stimulating unwanted responses to autoantigens

The book is well produced, and I noted few typographical errors Its usefulness might have been increased if the bibliography had been made to serve additionally as an author index, simply by adding the page numbers of each citation after each listed reference 'Adjuvant workers' should find this monograph worth buying

D W DRESSER

Natives of Canada

Athapaskan Adaptations By James W Vanstone Pp x+145 (Worlds of Man Cultural Ecology Series) (Aldine Chicago, April 1974) $7 50 cloth, $2 50 paper

THE Athapaskan speaking peoples occupy a vast forest region of northwest Canada and most of the land surface of Alaska As Walter Goldschmidt the series editor suggests in the foreword, Athapaskan culture was less dramatic than the Northwest Coast or the Plains types, but no less adaptive, "a cultural flexibility, a quiet competence, a sustained ability to blend in with local environment" (page vii) The Athapaskan groups are seen as small communities of hunters and fishermen adapted to salmon fishing in the Pacific drainage lowlands, collective caribou hunts in the open northern regions, and more solitary moose hunting in the dense forested southern regions of Great Slave Lake

The book is an attempt to present a very generalised account of the ecology and social organisation of the Athapaskan groups at the time of contact with European and Russian entrepreneurs about 1750 AD It is based on the current research of many scholars in the field, as well as those of the 1950s and the 1920s In addition there are two principal sources from the early period, namely Samuel Hearne's account of his trip to the Coppermine River, NWT Canada of 1769–72 and Henry Allen's to the Ahtna of the Copper River, Alaska in 1885

The linguistic units were divided into small, mobile task forces of hunters and fishermen, often widely separated if not isolated Economic variations from this pattern were minor But the author demonstrates an unaccountable variation of social practice Among the 'Northern Athapaskan' some units have hereditary leadership, others have none There is bilateral descent in some while others have matrilineal sibs, some have group and some have individual ceremonies There is both matrilocal and flexible residence patterns, and a modified male vision quest is present in some

Abandonment of the aged is present in most, but one region recognises special high status for the aged. Even the potlatch is present in some regions. Perhaps most significant is the range of marriage regulations, all the way from parallel cousin through cross-cousin to no-cousin marriages.

The picture of the Athapaskan before outside contact is obscured by the fundamental changes wrought by two hundred years of the fur trade, and one hundred years of Christian mission activities. Since the end of the Second World War, education, health facilities and police administration have resulted in the development of larger population centres, the construction of many buildings, and the presence of alien supervisors in both Canada and Alaska.

The author describes in detail the settlement of Old Crow in the Yukon which exemplifies this recent change. He suggests "the community members almost never join in any work for the community as a whole because there are no leaders who can organise activity for the general welfare and improvement of the settlements" (page 117). Thus the members of Old Crow "have not been successful in developing social mechanisms to cope with the problem of living in large, permanent settlements" (*ibid.*). Although this is a general phenomenon throughout northern Canada, the author indicates the cause of failure is that "traditional social organisation emphasised the freedom of the individual family and the authority of the family head" (page 118).

Surely to seek causes in traditional culture forms is an oversimplification at best. Rather, the large permanent settlements based on imported funding and direct administration by aliens have emasculated the indigenous people. Most of Canada's indigenous peoples are in a similar position to these at Old Crow and are equally politically inarticulate. Many observers feel that the only change possible will be through the new regional organisations of Indians, Metis and Inuit (Eskimos). Dr Vanstone's views on their organised protests and negotiations with governments would have been valuable.

R. W. Dunning

Structure of the cerebellum

Cerebellar Cortex: Cytology and Organisation. By Sanford L. Palay and Victoria Chan-Palay. Pp. xii+348. (Springer-Verlag: Berlin and New York, 1974.) DM156; $64.

For the neuroanatomists of the nineteenth century, the cerebellum was a source of fascination, providing an unending series of vexing problems and forming the battle ground between the neuronists, who believed that each nerve cell was a discrete entity, and the reticularists who argued that all the nerve cells in the brain were fused together into one extensive network. The reasons for the fascination and the conflict become quickly apparent to any reader of this superb book; six types of neurone, including the largest and the smallest types in the central nervous system, regularly interconnect to process the inputs conveyed by the two main afferent fibre systems. Furthermore, the modes of terminal arborisation of the fibres in the cerebellum exemplify both the simplest and the most complex types found in the nervous system.

During the latter part of the last century, the various cell types of the cerebellum were quickly recognised and, with the new staining technique, their processes were gradually characterised. All the great neuroanatomists of the past contributed to this achievement but it was Ramón y Cajal who in 1888 gave precise descriptions of all six cell types, as well as the 'climbing' and 'mossy' fibres, which form the two input pathways. But there remained a host of important questions which could not begin to be answered until the electron microscope had been developed.

This book takes up the problems Ramón y Cajal left unanswered. Starting from the Purkinje cell, the authors describe in successive chapters all the remaining cell types and afferent fibres. Each chapter begins with a brief historical account of the discovery and ensuing controversies, together with an assessment of the original descriptions. Then the up-to-date knowledge is lucidy presented. The book may be read at various levels. For the uninitiated camera lucida drawings of different neurone types and electron micrographs accompanied by detailed expositions of ultrastructural features will provide a glimpse of the basic 'wiring diagram', and of its extraordinary intricacy. For neuroscientists, it will be an invaluable source book. It provides a comprehensive overview of the important facts unravelled in the past two decades. These include evidence derived from both the optical and electron microscopes, as well as the electrophysiological techniques. In addition, numerous details of previously unpublished observations of the authors are incorporated.

This book is a credit to its senior author, whose sustained interest and imaginative application of current techniques have brought about a significant advancement in our understanding of the structure of the cerebellum. It is a worthy successor to the classic works of the Ramón y Cajal era.

Shin-Ho Chung

Uncontrolled eddies

The Analysis of Eddy Currents. By Richard L. Stoll. Pp. xii+128. (Monographs in Electrical and Electrical Engineering.) (Clarendon: Oxford; Oxford University: London, January 1974.) £3.50.

Despite the crucial importance of eddy current phenomena, in devices like the induction motor, the undergraduate introduction to the subject is necessarily very limited. It depends largely on equivalent circuit concepts, and assumes that the eddy currents are confined to insulated conductors, and are under the control of the designer. Recent developments in large, electrical machines and electromagnetic devices have added to the range of problems in which the eddy current flow paths are not so clearly defined or controlled, and have provided a fresh impetus to their study. In general, the problems demand a direct solution of the underlying field equations, yet even the simple methods and solutions receive scant attention in most textbooks on electromagnetic fields, and descriptions of more recent work are confined to the original references. Dr Stoll's book fills an important gap. It combines in a condensed and convenient form a description of basic analytical methods, and an introduction to numerical techniques by which the more complex problems, including flux and current penetration into iron, can be solved by computer. It is an essential addition to the bookshelf of those faced with the need to analyse eddy current problems, particularly at low (that is power) frequencies.

Dr Stoll writes as a specialist who has made considerable contributions to his subject, and he has included a great deal of useful material in 125 pages. Yet his treatment is admirably lucid, and the relevant parts of the book are well suited to the needs of electrical engineering and physics undergraduates and others seeking an introduction. The description of numerical methods is necessarily selective, in an area which is rapidly developing, and although it is a pity that topics such as finite-element and integral methods have had to be referred to only briefly, the material is well chosen for those whose principal interest is in applying the methods, rather than developing them. The book meets admirably its intended purpose, which is to provide an understanding of the more important methods and a bridge to specialist literature. It is well produced, and is a pleasure to read, although at some points a less condensed treatment, with additional diagrams, might be helpful to students with only a limited familiarity with electromagnetic field equations.

C. J. Carpenter

Mechanisms in minds

Current Biochemical Approaches to Learning and Memory. Edited by Walter B. Essman and Shinshu Nakajima. Pp. ix+205. (Monographs in Modern Neurobiology.) (Spectrum: Flushing, New York; distributed by Halsted (Wiley), March 1974.) £8.40.

This book consists of an introductory chapter by the two editors, and eight chapters devoted to current research in the area of biochemistry and learning. The first chapter is a short, readable and sensible historical review, and the only chapter for which there would seem to be any excuse for publication. The remaining chapters are brief, superficial, badly written, and poorly edited; mistakes in spelling and punctuation, and peculiarities of grammar are common throughout, particularly in those chapters by authors whose first language is, as is all too evident, not English. There is not even any consistency in the way in which references are set out, some authors giving titles of papers, and others, page numbers only.

The brevity of the chapters has two unfortunate effects; first, no attempt is made to place the research described in a wider setting, by showing its relationship to the often very large body of relevant material from other laboratories. Bogoch, for example has a reference list of 29 entries, 28 of which are to his own work; Tsukada has one reference (to Tsukada). In some cases, there is a distinct impression that the authors are simply unaware of the research carried out in other laboratories—the chapter by Ilyutchenok and his colleagues on cholinergic mechanisms does not refer at all to Carlton, and only in passing to Deutsch.

The other consequence of brevity is that the evidence in support of authors' claims is very rarely presented in sufficient detail to allow its evaluation, and frequently no very clear idea emerges of exactly what procedures were used. What little hard evidence is provided is distinctly discouraging, displaying as it does, in almost every chapter, a cavalier attitude to statistics. For example, Fjerdingstad, in the only chapter on transfer of learning, applies one-tailed *U* tests to each of several test sessions on the same animals, without any regard to the fact that acceptable significance levels change drastically with such a technique. Whether such considerations would influence the editors is open to doubt. Nakajima in his chapter reports an experiment, with six groups of eight animals each; although he first states that the number of animals is insufficient for statistical analysis, he nevertheless goes on to tell us that the results "suggest" one conclusion and "also indicate" another.

In short, this is a book that should never have been published. It is extremely expensive, and one can only hope that few institutions, and fewer individuals, will feel obliged to purchase it.

Euan M. Macphail

Plants in action

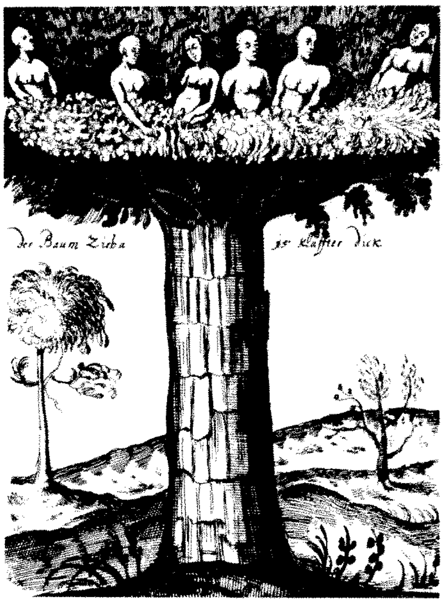
Dynamic Aspects of Plant Ultrastructure. Edited by A. W. Robards. Pp. xii+546. (European Plant Biology Series.) (McGraw-Hill: London and New York, June 1974.) £8.95.

Dr Robards has brought together seventeen distinguished workers to write fifteen chapters in his symposium. It is difficult to fault either the credentials or the international flavour of the chosen authors. The advantage of the symposial approach is that each section can be written at the front of knowledge of that topic and, given a firm editor which has here been achieved, a relative evenness of treatment is possible. My quarrels with statements made by individual authors are of the level to be settled at small conferences and not of major substance. The disadvantage of the symposial approach is that each author is constrained to write within a precise framework and this inhibits cross correlations or interpretative sections on, for example, overall control mechanisms or communication between cells. A book of this type, excellent as it is, must therefore be read in conjunction with single-author works at the same level.

Errors are few and good references numerous. Economies such as the lack of line 'justification' are justified and bother me not at all. But was it worth putting in some, only just relevant, expensive colour plates and printing all the rest of the plates on muddy matt paper even muddier than Dr Robards's previous text in this series? It does hold the price down to a reasonable level, but it must have enraged the authors of some of the beautiful micrographs included.

B. E. Juniper

Erotic tree



The Zieba tree, described in 1676 by Christopher Vielheuern. From *Bizarre Plants: Magical, Monstrous, Mythical* by William A. Emboden. Pp. ix+214. (Studio Vista, London, 1974.) £2.95.

Techniques for analysis

Analytical Chemistry: An Introduction. By Donald J. Pietrzyk and Clyde W. Frank. Pp. xx+667. (Academic: New York and London, April 1974.) $13.95; £6.70.

The course described in this book was originally designed as a one-semester introductory course in analytical chemistry for subsidiary students, but it is now intended for students taking chemistry as their major subject. There are four main areas: spectroscopy, acid-base methods, potentiometry with special reference to ion selective electrodes and chromatography. These topics are subdivided into further parts.

Analytical chemistry now covers such a broad area that only in specialised courses can it be treated in depth. An introductory course should set out to show what techniques are available and provide some guidance as to which are suitable for a particular purpose. Generally, in an introductory course the main limitation is time and therefore a very careful selection has to be made.

The authors of this book have obviously given a great deal of thought to the problem, for this is a well balanced course presenting information on all the basic methods and techniques of analytical chemistry. A balanced picture of the respective parts played by instrumental and classical analysis is also provided.

Some excellent exercises are described and possibly the only criticism to be made here is the omission of one on solvent extraction; it is a curious fact that most teaching texts on analytical chemistry avoid this exercise, despite the importance of the subject. Another criticism is that a better selection of redox indicators might have been provided; the quoted potentials of some are incorrect.

These are very minor shortcomings and in general this is an admirable and well thought out text which all teachers of analytical chemistry should find of great value.

R. Belcher

See-through women replace yellow-bellied sapsuckers

Boston's Museum of Science Open daily Adults $2, children $1 Closed Thanksgiving, Christmas, New Year, July 4, Labour Day

THE Museum of Science in Boston lives in one of those bleak, deserted areas peculiar to American cities Although a mere five minutes' walk from the city centre the pedestrian finds himself hideously outnumbered by speeding cars On a causeway across the Charles River, the buildings tempt you in by their very humanity amidst the heartlessness, and (again an American feature) an affluent and joyful interior in stark contrast to the external world For the museum, whatever else it is, is full of joy, and the most un-museum like museum I have yet seen

One colossal advantage it has over many other museums is its youth When Bradford Washburn became director in 1939 it was sited in a four-square civil-war vintage building in downtown Boston, and as with so many museums of science, it was hard pressed for space and dominated by natural history exhibits Washburn, a distinguished explorer and mountaineer, returned after war service to create a completely new multidisciplinary museum that would be particularly attractive to the young In many ways he was lucky The forties and early fifties were times when it was probably easier than today to persuade donors that science needed good publicity Harvard Museum of Comparative Zoology, furthermore, served as a reference museum, releasing him from the burden of endless display cases of stuffed animals The closeness of many educational, medical and technological institutions ensured ready assistance and advice (though is this true in London, say?) And there is Boston—just the right sort of city to plant this sort of thing in

But if he was lucky, he has also had an incredible flair for what appeals to the young, and above all what makes them feel at home As part of my preliminary research I took a couple of young boys—5 and 3—and let them loose They found a lot that they enjoyed—a surprisingly large fraction of the exhibits—but eventually they gravitated to a huge tyre hanging, playground-style, from a ceiling This gave them twenty minutes of simple fun, with them happily parked therein (indeed eventually almost impossible to prise out) I could look around at leisure The next day, when I met Ned Pearce, the Museum's librarian, he had marked on a map for me what the most popular exhibits were—an uncannily accurate description of Wilson's and Philip's somewhat erratic route through the museum And the giant tyre? Yes that was on the list and Mr Pearce was mildly defensive about it, thinking I as a high priest of scientific orthodoxy would be cynical about a mere plaything "You see we regard it as an important exhibit—children learn that there are things they can climb on here and not get screamed at One of our key roles is to persuade kids that this a friendly place and that people here are nice " This is put somewhat more sentimentally in one of their glossy brochures as "When [children] arrive they are not only met by wonder and excitement and discovery They are also met by love " Mawkish stuff maybe but there is more than a grain of truth in it A substantial fraction of the three quarters of a million visitors each year come in school buses many of these from the inner city They are naturally hostile to anything that smacks of authority and schoolroom formality, and for them a large number of volunteer guides—adults or teenagers—are laid on It is essential, says Pearce, that guides, whether volunteer or full-time, should not be seen as policemen

There has been much discussion in the museum on the social aspects of the educational programmes that they run, which include Saturday morning classes Is the museum seen as a middle class institution perpetuating science as a middle class occupation? Questions like this (barely asked at all in England) are put in sharp focus when a busload of inner-city poor debouches into the arms of a potentially loving phalanx of suburban moms with their biology degrees and their worthy aims As far as possible the museum tries to recruit volunteers and guides who can relate at first hand with the underprivileged but it is a difficult task Already they are phasing out some of the Saturday activities which have tended to become high quality baby-sitting for the middle classes

Once inside, how does the youngster experience the "enjoyment of learning" that Washburn dedicates the museum to? I think he does it in two ways—through the imaginative use of space, and through unashamed compromise As museums go it has a very average amount of square footage per visitor But there seemed to be less clutter and less pressure to circulate Come on a rainy Sunday, said Pearce, then you'll see things differently, but I think the point is that if you go on a sunny Wednesday you can really breathe

And the compromise—it's not a poor man's university You can mingle with a full sized *Tyrannosaurus rex* but the next exhibit along is a giant magnet one way, and a telephone exchange the other The hot air balloon (for my money the best exhibit) rubs shoulders with the deep diving submarine There is no logical order or historical succession to it all, it is a huge sampler Washburn says "To touch a snake, to see liquid air or to pat a porcupine are never-to-be-forgotten experiences which will launch a youngster into a science career a thousand times more rapidly and powerfully than any number of carefully labelled exhibits"

In twenty years how has fashion changed in Boston? Natural history was and is unassailably Number One at all ages, declares Washburn But whereas the prime interest used to be birds it is now 'me' the human being and how it works Certainly the transparent woman, the stages in childbearing, the heart and the appendectomy draw more than such commonplaces of the seventies as lunar modules and computers, though the number who stay the course of ten realistic stages of removing an appendix without retiring slightly queasy is small

The museum runs on an annual budget of around $2 million of which about half is collected through admissions (including state-paid visits of schoolchildren) and memberships Private and corporate donors find the rest Of course there is debate about the admission charge, although the staff fear for the crush on Sundays were entrance to be free But at present that cannot even be considered

Washburn has been successful at persuading the corporate donors to pay without visible returns The museum is mercifully free of brand name exhibits which elsewhere range from the excellent, such as the IBM mathematics pavilion at Chicago, through the blatantly commercial "would you mind stepping into our exhibit and answering a five minute questionnaire on your cooked-ham eating habits?" also at Chicago to the dreary cutaway models of factories made by the apprentices and presented by the managing directors at—well, never mind where

As long as Brad Washburn and his staff can continue to say 'no' if it isn't fun, I suspect that Boston will continue to be a hotbed of bright young scientists, many of whom will have experienced their first awakenings in or near the giant tyre DAVID DAVIES

obituary

Léon Rosenfeld

LÉON ROSENFELD was born at Charleroi, Belgium, on August 14, 1904 He studied physics and mathematics at the Université de Liège where he obtained a D Sc in 1926 He was associated with L de Broglie and P Langevin in Paris (1926–27) with M Born in Gottingen (1927–29) and with W Pauli in Zurich (1929–30) During this period he also collaborated with Th De Donder in Brussels In 1930 Rosenfeld visited Copenhagen, where he began a collaboration with Niels Bohr which lasted until Bohr's death in 1962 Rosenfeld joined the Université de Liège in 1930 From 1940 to 1947 he was Professor at the University of Utrecht and from 1947 to 1958 at the University of Manchester In 1958 Rosenfeld became a professor at NORDITA

Léon Rosenfeld was a member of the Royal Danish Academy, the Académie Royale de Belgique, the Académie Internationale d'Histoire des Sciences and the Académie Internationale de Philosophie des Sciences He received the Prix Francqui, a high Belgian distinction, in Brussels in 1949

The scientific interests of Léon Rosenfeld were unusually wide They included quantum electrodynamics, nuclear physics, statistical mechanics, epistemology and history of sciences His monographs *Nuclear Forces* (1948) and *Theory of Electrons* (1959) had a wide circulation

His best known contribution is probably his fundamental paper with Niels Bohr in 1933 on the measurability of electromagnetic quantities In this paper Bohr and Rosenfeld showed that the only meaningful statements of quantum theory when applied to electrodynamics concern averages of field components over finite space time regions In this way they proved the logical consistency of quantum electrodynamics

Another series of well-known papers by Rosenfeld, mostly in collaboration with Humblet from Liège, concerns the theory of nuclear reactions (1962–65) These papers introduced a new and precise definition of resonant states

All his life Rosenfeld was deeply interested in the foundations of physics The 'Copenhagen' interpretation of quantum mechanics, still accepted by the majority of physicists, was developed by Bohr and Rosenfeld following the pioneering work of Heisenberg, Born and Bohr

After Bohr's death Rosenfeld appeared as his natural spiritual heir It would be a mistake, however, to consider Rosenfeld as simply the guardian of the Copenhagen orthodoxy More than anyone else he knew that one of the main objects of theoretical physics is to define the precise domain of validity of concepts involved in the mathematical models of physical reality He wrote, "No physical concept is sufficiently defined without the knowledge of its domain of validity" For this reason he devoted most of his efforts during the last years of his life to showing that the coherence of the rules of quantum mechanics can be tested explicitly through the study of the dynamics of large systems For him the foundations of quantum mechanics had not to be looked for in some axiomatic formulation valid for small systems with a discrete spectrum energy but, on the contrary, in the study of the limiting procedures involved in going to large systems Since his early work he emphasised that there should exist a new type of complementarity between the "irreversible character of the macroscopic behaviour of the large systems and the time reversal invariance of its dynamical description at the atomic level" The outcome of these considerations is a generalised transformation theory which he developed in close collaboration with Prigogine's group in Brussels and in Austin This transformation theory makes it possible to go from the usual representation of dynamics in terms of the Liouville–von Neumann operator *L* to a new representation in which the contributions responsible for irreversible processes are explicitly displayed

His fatal illness brutally interrupted his contribution to the promising new development in theoretical physics

For Rosenfeld science was a total engagement Far from being a simple intellectual curiosity, Rosenfeld, like Bohr, "conceived science as a human endeavour to achieve harmony with nature, a common task to which it was the earnest duty of each individual scientist to contribute his share"

Rosenfeld will be remembered and missed by all his friends and scientific collaborators who had the privilege of knowing him and of appreciating his exceptional personal, moral and intellectual qualities

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Announcements

Appointments

M. A. Sleigh has been appointed to the chair of biology at the **University of Southampton** (corrected announcement)

K. C. Calman has been appointed the Cancer Research Campaign Professor of Oncology at the **University of Glasgow.**

International meetings

September 10–11, **Energy Conservation Conference,** Glasgow (National Engineering Laboratory, East Kilbride, Glasgow G75 0QU)

September 23–26, **International Symposium on Contamination Control,** London (The Sam Black Organisation Ltd, 4 Harmsworth Way, London N20 8JU)

September 24, **Symposium on Human and Veterinary Trematode Infections,** London (Dr A B Simmonds, Chelsea College, Manresa Road, London SW3)

October 1–3, **1st National Topical Meeting on Nuclear Process Heat Applications,** New Mexico (Robert Y Porton, Public Relations Office, Los Alamos Scientific Laboratory, PO Box 1663, Los Alamos, New Mexico 87544)

October 21, **Chemical and Mechanical Energy—Utilisation in Agriculture** (Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London SW1X 8PS)

Erratum

In the article "Ionisation ledges in the equatorial ionosphere" by R Raghavarao and M R Sivaraman (*Nature*, **249**, 331, 1974) the first sentence of the third paragraph should read, The cusps were observed on ISIS–2 ionograms from the dip latitude region 9°N to 7 5°S recorded at Ahmedabad on October 6, 1972

The reference number for equation (1) should be 5 instead of 4

nature

Volume 251 September 13, 1974

Can, or should, the swing be stopped?

A MONTH ago we looked at the way that student preferences were changing in Cambridge Now the Universities Central Council on Admissions has released a comprehensive statistical analysis of students' applications and admissions for all universities of the United Kingdom (Statistical Supplement to the Eleventh Report, £1 25) The figures apply to the year of admission 1973—it is some disappointment that it takes almost a year to produce a document of such importance—but the sections of most interest deal with trends since 1969

Many of the figures are rock-stable from year to year In 1969 11 6% of applicants came from the North West, in 1973 11 3%, 33% from the South East in 1969, 33 7% in 1973, and so on The percentage accepted is uniformly 51% both in space and time The only deviation seems to be Scotland which apparently can only get 40% of its applicant sons and daughters into universities But then your instinct says that surely the Scots do not seem any more stupid than the rest, further hunting reveals that 'three Scottish universities are partly outwith the UCCA scheme' and your dictionary tells you what that word means and that it is of Scottish origin

The middle classes, less than 30% of the population, provide almost three quarters of the candidates—and working class applicants have declined from 29% to 26% in five years, although they do now fare marginally better in getting accepted

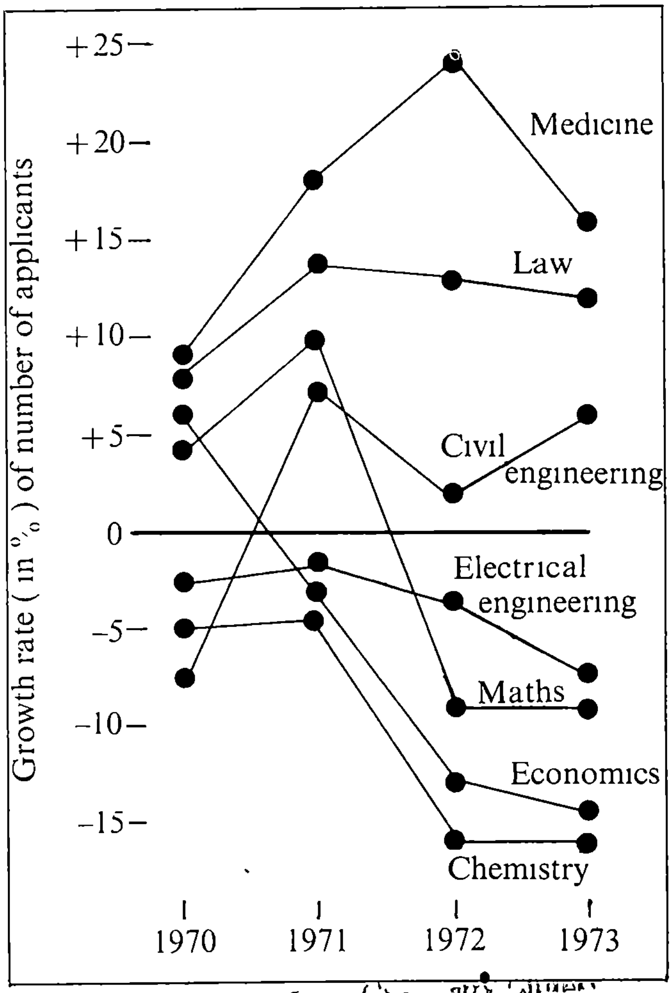
Whilst all these trends are slow or negligible, one trend has been dramatic the swing away from science In the figure we show the way in which fashions have been moving in seven subjects which have large enrolments at university For instance, a value of +10% for mathematics for 1971 means that there were 10% more applicants for entry in 1971 than in 1970 who gave mathematics as their preferred subject Some other subjects not shown here have comparable trends, physics and mathematics follow similar patterns, so do mechanical and electrical engineering Geography, English and History are relatively static Social, administrative and business studies follow strange ups and downs but have shown no long term trend

In terms of absolute numbers, there were 7,000 applicants for medicine in 1969, almost 13,000 in 1973, 5,000 for law in 1969, almost 8,000 by 1973 Chemistry on the other hand had slumped from 3,700 to 2,400 Less than one in four of those applying in 1973 to do medicine were admitted, whilst two thirds of those applying for physics or chemistry were accepted What is more a substantial fraction were admitted to physics or chemistry who had not even listed it as first choice

It used to be said disparagingly of medical students that they were only in medicine because they found physics and chemistry too difficult, but figures in the UCCA compilation show that the increased selectivity now possible has made the entering medical undergraduate the best academically qualified of any

Everyone will have his own explanations of these trends, but two things need careful underlining The dramatic transition from the 1971 to the 1972 entry was no flash in the pan since the 1973 growth rates are closely similar to those of 1972 If science applications were depressed in 1972 by gloomy job prospects (up to six or seven years in the future!) and disenchantment with technology's environmental record, then to keep the growth rate so negative for yet another year would have required even more gloom and disenchantment.

Second, it is insufficient simply to acknowledge the falling off in science applicants, say yes it was bound to happen some time, hold a meeting or two and hope that the problem resolves itself somehow The diversity of opinions expressed at the British Association last week (page 91)—good prospects in engineering, bad in biology cannot but confuse the school-leaver The message from school science teachers is that there is no turnaround in recruitment to science in the immediate future. Can the country, can science afford to lose out in this way? The answer is not automatically that it can't, and it would be foolish blindly to assert that the number of scientists must always grow Nevertheless at present the size of the annual intake is controlled by forces which are so remote from job prospects four to seven years hence that a market place type of situation could hardly be said to exist Eventually the community must come to grips with the question of how it wants its future size determined



[illegible]

Ominous news for marine science

The Law of the Sea Conference is over and the way in which marine scientific research is to be organised and controlled is not yet agreed

CARACAS—the site of the Third United Nations Conference on the Law of the Sea—a booming, noisy city, set amidst the green hills of the national park area Ten weeks of slow and protracted discussions in three main committees, each open to delegates from all the 137 countries attending, plus numerous intergovernmental and nongovernmental bodies of all sizes, composition and pursuasion Superb arrangements laid on by the Venezuelan Organising Committee, together with the United Nations Secretariat (at considerable cost) though a number of nations (particularly in Africa and Asia) expressed concern at the communication difficulties they have encountered and expressed their need to keep in close touch with their governments, particularly during the later negotiating stages of the conference Chiefly for this reason it was decided to hold the next session in Europe

Almost unanimous agreement that further sessions should not exceed six or seven weeks in length as humans get tired and stale if they attempt to confer for a longer period and little is achieved in the extra weeks Winter troglodytes from northern latitudes trying to avoid another summer session —we have not had an opportunity to see our families in the sun for six years, smaller and less well off countries trying to reduce the length of future sessions, owing to the high cost of keeping large delegations abroad—countries have now had the opportunity to define their positions, let us get on with the negotiating and this will only happen during the final session, other countries talking about two sessions in 1975 and at least one in 1976

Countries with differing views on the importance of the whole exercise—some suggesting that this was the most important conference that has ever taken place on the earth, and that we were trying to save civilization, others wondering whether the conference had the slightest relevance to the future of the human race, and still others believing sincerely that it was doing positive harm to the aspirations of the less developed countries

Sharp disagreements within delegations, usually ending up with victory for military and economic interests over those of the marine scientists and environmentalists Different types of disagreement between marine scientists —one faction claiming that freedom of scientific research in the oceans is essential for the future of scientific research as a whole, and that the restrictions proposed would either kill marine science dead or make it an undercover activity which no individual state or international authority could hope to prevent, or the opposing view—that developed countries have got to realise that the days of 'Challenger' type expeditions at the expense of the natives are over, and it is now the turn of the developing countries

These may be negotiating positions, of course, and doubtless if the declaration made by the President and endorsed by the conference on June 27, is to have any effect, the extreme views will disappear in the interests of gaining concessions elsewhere This declaration reads

"Bearing in mind that the problems of ocean space are closely interrelated and need to be considered as a whole, and the desirability of adopting a Convention on the Law of the Sea which will secure the widest possible acceptance, the conference should make every effort to reach agreement on substantive matters by way of consensus and there should be no voting on such matters until all efforts at consensus have been exhausted "

This consideration is in fact vital when it is remembered that the first UN Conference on the Law of the Sea, Geneva, 1958, which produced four international conventions, has received relatively poor support from states These were the Convention on the Territorial Sea and Contiguous Zone (46 states parties, in force since 1964), the Convention on the High Seas (54 states parties, in force since 1962), the Convention on Fishing and Conservation of the Living Resources of the High Seas (35 states parties, in force since 1966), the Convention on the Continental Shelf (53 states parties, in force since 1962) Furthermore, the second UN Conference on the Law of the Sea, Geneva, 1960, failed in its attempt to resolve disagreements over the breadth of the territorial sea and fishery limits

The three main committees inherited the work of the UN Committee on the Peaceful Uses of the Sea-Bed and the Ocean Floor beyond the Limits of National Jurisdiction and it is on the lengthy deliberations of that committee over a period of five years that the present discussions are based and the texts of the convention articles will be developed

It is becoming clear now that the final agreement will most likely include a territorial sea twelve miles in width, an economic zone beyond the territorial sea (referred to by the Latin Americans as the 'patrimonial sea') and an international zone covering the deep sea-bed, the water column being high seas The general trend embodying several proposals is that countries where the width of the shelf is less than 200 miles, will have an economic zone of 200 miles covering both the shelf and the water column, others, where the shelf extends to over 200 miles, will have an economic zone of 200 miles and in addition sovereignty over the sea-bed out to the edge of the continental rise (this additional sovereignty will not include the water column) The fact that this limit is unidentifiable in most parts of the world because of the lack of data or lack of rise, has so far been conveniently ignored

Among the more controversial matters on which as yet no agreement is in sight, are the extent of coastal state jurisdiction in the economic zone with regard to such matters as pollution control, scientific research, exploitation of living resources, and the degree of control to be exercised by the international authority over the international sea-bed area—this varies from the "none" of the states most physically capable of working in this area to the vesting of exclusive and direct control of all exploration and exploitation in the International Authority A serious draft article "Right to undertake Marine Scientific Research", at present on the record, states "Marine scientific research in the international area shall be conducted directly by the international authority and, if appropriate, by persons, juridical or physical, through service contracts or associations or through any other such means as the International Authority may determine, which shall ensure its direct and effective control at all times over such research"

The fear of scientific research shown by many of the developing countries is based on two factors, one real and one controversial The former is the military bogey, the possibility that a ship operating off the coast of another state may be gathering data inimical to the coastal state, in addition to its declared programme (and not necessarily oceanographic data, for example the

Pueblo episode has been invoked on more than one occasion). In fact this is a case of the past behaviour of certain countries with regard to the release of oceanographic data coming home to roost. The theory behind the present World Data Centre system is that each country by injecting its data into the system qualifies to extract all data sent in by other countries. In fact a number of countries are notorious in that they participate in the system but, caught by military classification of data, they submit a minimum of their own, at the same time however calling loudly for complete submission of all data by others. This is particularly true of water temperature and to a lesser extent salinity data, of use to the military for acoustic detection of submarines. Marine pollution data, being of little military value, escapes the military clampdown.

The counter arguments deployed are that no convention or international regime is going to stop this type of behaviour. The navies of the world will continue with this policy until submarines or sonar become obsolete and it is the marine scientists wishing to work on other pressing problems who suffer.

The second fear is economic and is based on the premise that knowledge is power—in some unexplained way knowledge of the sea or sea-bed gained by a country carrying out scientific research, can be used to its economic advantage at the expense of the coastal state. In fact the coastal state desperately needs such knowledge if it is to benefit from any worthwhile exploitation of the area over which it has rights, and illegal exploitation which is a much slower process, will be far easier to prevent, particularly if the International Authority were developed to this end and the world's navies, which after all are the only possible forces that can police these areas, are mobilised in support.

At the present, the International Authority is seen by many countries as a panacea for all ills. There has been complete lack of consideration given to the way in which the authority will carry out its terms of reference and the structure it will need for this purpose, that is, how it will assess research projects submitted to it, whom it will recruit to carry out this task, what they will be looking for anyway. In theory, the authority will have to study each project submitted to it and to decide that the declared objective or purpose of the research is as stated, that it is not to the detriment of any coastal state (if in waters over which it has right) and that other criteria are laid down are being adhered to.

To undertake a task of this nature, a very large staff of highly qualified marine scientists of many disciplines would be needed and it is quite clear that inherent delays in obtaining consent would be enormous. At the same time that the developing countries are asking for the international authority to take on this vast and sophisticated task, they are clamouring for a transfer of technology, by which is meant an expanded training and education programme to build up their national marine science infrastructures. This latter exercise is fully justified but could well be killed by the former. But in any event very little detailed or serious consideration has been given to this subject and the conference has before it only one very general proposal dealing with the matter.

The basic world shortage of marine scientists in all but a few countries has led to a considerable reluctance by the United Nations and its specialised agencies to recruit top people in this field from the developing countries, as this usually results in an immediate weakening of the marine science infrastructures in the countries concerned; almost certainly these have been painstakingly built up over a number of years.

"It is a sad reflection on our times that . . . most delegations present consisted of lawyers and politicians, with little or no scientific support"

The staff, for a viable international authority capable of carrying out the assessment tasks proposed for it, would have to be obtained either from the very developed countries whose activities the authority has been set up to restrain or if, as is more likely, the United Nations staff quota system is used, largely from the developing countries; the latter arrangement would spell disaster to the marine aspirations of many of those countries.

There is also a real fear that the international authority will become yet another United Nations agency, to be paid for by the developed countries, that is, those countries whose activities will be most heavily constrained, with a complete lack of internal policy except to increase its empire and to become operational at the expense of other bodies which have been working in the same field, often for many years. Existing United Nations bodies already have a good if inadequate record of activity in this field; the Intergovernmental Oceanographic Commission of Unesco, for instance, already offers its member states an independent assessment facility for any project in which there is cause for dispute between a coastal state and a state wishing to carry out scientific research in waters over which the coastal state claims jurisdiction.

A fruitless exercise at present being carried out by certain of the developed countries, is an attempt to legislate separately for pure and applied research, as if these are rigidly definable activities. Certainly there is a white area of pure marine scientific research such as investigation of the history of the earth and the theory of plate tectonics; certainly there is a black area of applied research such as development of seismic survey methods to detect salt domes capping oil reservoirs, thus providing an indication to the oil companies of the most likely positions in which to drill to obtain a strike; but in between, there is a great grey area having both pure and applied connotations inextricably mixed, and anyway there is very little pure research that does not have an applied fallout of some kind. Besides this, such legislation would do considerable harm to applied research and survey work, needed by both developed and developing countries alike if the ocean resources are to be exploited economically for the benefit of mankind.

An unfortunate trend that has evolved during the Sea-bed Committee and conference sessions, has been the apparently successful attempt to lump all sorts of diverse data collecting activities under the heading of scientific research, which of course they are not and never have been; these include meteorological, oceanographic and bathymetric data gathering for such purposes as weather forecasting, compilation of navigational charts and so on. For instance, if a country wishes to place automated transmitting buoy systems (known as ocean data acquisition systems, aids and devices, or 'ODAS') off the coast to report the approach of hurricane force winds, this is now known as scientific research.

It is essential that such activities should be seen in their true perspective as ocean services—activities for the compilation of products for the benefit of developed and developing countries alike. Restrictions on data collection and exchange can help no one and do harm to many.

It is a sad reflection on our times that although, as pointed out by the Secretary of the Intergovernmental Oceanographic Commission to Commitee III, a basic knowledge of the oceans—what they are, how they behave, what they consist of, and so on —is essential to the success of the conference and the subsequent convention, yet most delegations present consisted of lawyers and politicians, with little or no scientific support. ☐

international news

The British Association at Stirling

THIS year's summer meeting of the British Association (BA) in Stirling, four days of lectures devoted to science ranging in purity from the geology of Scotland to witchcraft, was deemed a great success by its retiring President, Sir John Kendrew. More than 2,000 members attended this unique forum, the best attendance for seven years.

Dr Magnus Pyke, Secretary of the Association and Chairman of Council, (pictured above) sees the BA as a "peripatetic university; it obtains its strength from movement". Certainly, to judge from those in attendance, it seems to recruit many of its officers and much of its following from towns which have been host to the association in the recent past. The ambience does seem to be very important and was a major factor in the success of this year's venue, as a result of "the intellectual richness of Scotland".

There was no feeling of nostalgia for the BA meetings of the past, at which many new results were communicated. Sir John made it clear that though original work would be very welcome, "the process of communicating science has changed completely since the early days of the BA". Dr Pyke feels that the function of the BA is to communicate a policy of social responsibility. Dear to Dr Pyke's heart are the working parties and their reports. But (as the reports reflect) Dr Pyke is adamant that the association should not adopt a radical role. "We don't want to tear down ICI" he told *Nature* vehemently. "We are hear to make the facts clear; period." He stressed that it is the aim of the BA to educate members of the public so that they will be placed in a position from which they can exercise their rights as informed voters.

But it is interesting to consider whether those present really constituted a random section of the non-scientific public that Dr Pyke is trying to reach. The meeting, and the publication of the report on Biomedical Advances that had preceded it, received a fair amount of press coverage and Sir John felt that there had been an encouraging amount of responsible reporting. Without doubt the BA has been actively promoting itself in the recent past and at last seems to have realised that it is the only way that it can reach the wide public audience with whom it claims to communicate.

There was certainly an age gap in those attending the meeting. Most were either under 25 or over 50 and the small middle-aged group of active scientists seemed to be there to organise or to speak. Schoolchildren seemed much in evidence, both organised and disorganised, and some of the more fortunate undergraduates and schoolchildren had managed to get grants from their local education authorities. In addition of course there were teachers, government and industrial scientists (with a sprinkling of academics) and the mild elderly ladies who go each year simply for enjoyment.

In fact, schoolchildren seem to be the part of the population which the BA reaches most effectively. Young members are joining local BAYS groups at a rate which is beginning to cause administrative headaches, and Dr Pyke wryly admitted that the situation was becoming embarrassing. Many members of Council, notably Sir Lincoln Ralphs, are enthusiastic about the interest shown by the young, but Dr Pyke himself has been criticised by both the youngsters themselves and by many BA officials, for his lack of interest in the juvenile membership. There was certainly little provision for them at this year's conference.

Whilst it must be admitted that Dr Pyke, in his role as Chairman of Council, has provided an immensely important element of continuity in the organisation of the BA, it is sad that his enthusiasm does not seem to extend beyond his individual view of the current welfare of the association. There will come a time when, like it or not, Dr Pyke and his generation will have to leave and it would be as well to prepare for that contingency. It would be a pity if, after the successes of the current resurgence, the BA has to spend yet another period in the doldrums for lack of encouraging their future membership now.

Job crisis or manpower crisis?

by Eleanor Lawrence

If you want to be a research biologist, a degree can be a hindrance, according to Professor Kenneth Mellanby, former Director of Monks Wood Experimental Station. Addressing the British Association, he said that promising school leavers now stand a better chance than the average graduate of a career in biological research.

The career structure of research institutes, especially those financed by government, favoured two types of scientific worker. There would always be room for the first-class graduate with a higher degree, although the number of such vacancies, already small, may decline even further as the Fulton proposals improving promotion prospects for junior staff come into force. At the other end of the scale, said Professor Mellanby, most Directors prefer bright school-leavers for junior research and technical posts, and there is no lack of these wishing to take up a biological career.

Although Professor Mellanby acknowledges the situation he is far from happy about it. If good graduates and postgraduates cannot get jobs in research the quality of research will inevitably suffer, he said. In his view, the scientist who had risen through the ranks, admirable though he or she might be at the job, would not have a similar capability for research.

Professor Mellanby's impression was of a "great over-production of graduates and doctors and high unemployment". But, in fact, after an initial disappointment, the biology graduate usually found a congenial niche in a variety of other occupations.

But industries requiring graduates with a specialised applied training such as chemical engineering or metallurgy are experiencing a shortage of trained graduates to fill an ever increasing number of jobs. In 1974 the number of applications for degree places in chemical engineering dropped dramatically at a time when the demand for chemical engineers had suddenly taken a rapid upwards turn. The number of jobs in chemical engineering advertised in the Institution of Chemical Engineering's 'Diary' had dropped from 742 in 1969 to 178 in 1972 but had risen to 405 in 1973 and in 1974 was expected to top the 800 mark, said Dr David Sharp of the Institution.

Was this decline in graduate applications simply a result of straightened job prospects during the early 1970s? Dr Sharp is convinced that the cause lies much deeper; physical science teaching at secondary school level is neither inspiring enough nor of sufficiently high quality to encourage the school child in this sort of career he said. No graduates with engineering backgrounds went back into teaching at secondary school level, so that schools careers officers were completely ignorant of courses and career prospects. The tiny proportion of physics graduates entering teaching also worried Dr L. Cohen of the Institute of Physics. Chemistry may fare better, as in 1973 about 15% of first degree chemistry graduates entered teacher training.

Industry would no doubt find ways of getting round the specific manpower shortage, said Dr Sharp. The slack would probably be taken up by people trained in related disciplines such as chemistry. But a shortage of metallurgists could hold up programmes of metal reclamation and recycling said Dr D. C. Moore, President of the Institute of Metallurgists, as university departments of metallurgy declined and in some cases disappeared.

So the "job crisis" tends to look rather different depending on where one is standing. A picture emerges, not so much of a crisis, but of increasing diversification amongst science graduates. Everyone was agreed that, except in the special cases of the applied fields, there certainly has been a drop in jobs which use scientific training directly. Some parts of industry had increased their recruiting this year, but in the present financial situation, the universities and the scientific civil service would take fewer and fewer people.

But the patterns of first year employment seem to be evolving to meet the changes quite successfully, though how much individual disappointment is hidden behind the statistics is hard to tell.

Diversification is not necessarily a forced choice. Salaries of professional scientists compare very unfavourably with their contemporaries in management or administration in both industry and the civil service. Dr R. E. Parker of the Royal Institute of Chemistry produced comparisons of salaries between research, technical and management grades in the chemical industry which clearly direct the financially ambitious away from pure science.

Employment or lack of it amongst science graduates must be set in the wider economic context. Dr Cohen emphasised that absolute unemployment amongst science graduates was still lower than amongst graduates as a class. And it was pointed out more than once that other graduates had always been in the position of having to find work unrelated to their specific training.

Only connect

by Gillian Boucher

Science undergraduates lack the critical training needed to see their subject in the broader context of society or even of science as a whole. This is the thread running through the many experimental curricula and proposals for change described during a week-long session at the BA. Motives and methods varied widely but the courses described fitted into two broad categories: traditional science taught alongside 'science studies' (the salad principle); and the interdisciplinary science course (the porrage or porridge principle).

Dr David Edge, Director of the Science Studies Unit in the University of Edinburgh, described the alternatives to the traditional British subject-oriented course. First, a course aiming to fit the student for a career. This might entail teaching an additional subject like economics, or a skill like decision making. Second, the course could be more geared to the personal needs of the student who may feel that his development requires more than a single science taught in a particular way. Third, courses could aim to make students of more immediate use to society as a whole.

There was complete agreement that the traditional science courses and subjects should remain on the curriculum. But there was strong support for the provision of alternative courses. The commonest reasons given were the interests of the student's future career and of society, the two often being treated as identical. In the eyes of Professor F. R. Bradbury of the University of Stirling the 'Advancement of Science' was no longer so much in the public interest as it had been when the British Association was founded: instead, students should learn to manipulate the scientific knowledge already attained and apply it to the messy problems of real life.

Those who preach interdisciplinarity are adamant that the porridge should be smooth. It is not enough to teach several subjects side by side as in a joint honours degree or the old general degrees. It is not even enough to allow the student a great deal of choice between course units. Integration requires a totally new approach—an emphasis on the connections between subjects, which will lead to the subjects themselves being taught differently. To create such a course is very difficult—Dr Edge stressed the danger of a superficial juxtaposition of subjects. This can only frustrate the thoughtful student who wants to read further and discovers no core of knowledge and research below the superficial link.

Most of the interdisciplinary courses in existence in the United Kingdom—for example those at Aston and Stirling Universities and the Middlesex Polytechnic—teach two or more traditional subjects and attempt to link them by means of problem-solving courses. In these students are presented with a real life situation and asked to consider all the relevant knowledge from the various disciplines in coming to conclusions. Thus the student develops a skill which is not demanded by a traditional course, and is presumably more personally fulfilled and more ready to pursue a career and be of use to society. Sometimes even more is asked of these subject-spanning courses—they are supposed to provide the basic framework for the study of the traditional sciences. Thus chemistry and cell biology are taught in the context of a marine pollution course at Stirling. Clearly the links in many cases are fragile but perhaps for some students the applicability to social problems is enough.

There are other dangers of which, says Dr Edge, the proponents of the new courses may not be fully aware. In a subject-oriented course with rigid boundaries between subjects, the teacher is in firm control of what the student learns and can withhold from him the realisation of the gaps and ambiguities in knowledge until near the end of the course. All this makes for a very clear relationship between teacher and taught, with the teacher in charge. Changing to an interdisciplinary course will remove subject loyalties and may disorient both teacher and student. The teaching of a subject as part of an interdisciplinary course will entail teaching its fundamental concepts at a much earlier stage, and omitting much of the detailed knowledge of the subject. This will tend to diminish the traditional status of the teacher which again will be disorienting to all concerned and will be fiercely opposed.

Some of these problems have already been encountered by those teaching the new courses. Dr Edge's views on the relative power of student and teacher would not be accepted by all—seemingly Professor Jevons of the Department of Liberal Studies in Manchester feels that the student doing a course on marine pollution was in fact far more at the mercy of his chemistry teacher than a student doing a traditional chemistry course. The problem of subject loyalties may be solved by providing teachers and students of interdisciplinary courses with an institution which has some of the trappings of

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Scotland's future in the rocks

by Allan Piper

SCOTLAND'S economic salvation need not be entirely dependent on oil.

The quarrying industry in Scotland could soon be in a position to gain a substantial share in a market worth £2,000 million a year, which could have a considerable impact on the British economy, it was claimed in a lecture to the British Association. "It is fashionable to look on oil as the saviour of our economy", said Dr Colin Gribble, a geologist from Glasgow University and Mr Ian Wilson, an executive with Amalgamated Quarries (Scotland) Limited; but they claimed that, with the adoption of a scheme entailing the low-cost transportation of bulk material, high quality granitic material could become as important as oil in the longer term, both on the home and export markets.

Quoting government statistics, they said that by the end of this decade the annual demand for roadstone and construction material in Great Britain will have risen by 50%, so that an extra 100 million tonnes of aggregate will be required each year. The increased demand is likely to be particularly high in south-eastern England, a densely populated area already using a high proportion of the British annual production. Materials there are either very scarce or completely exhausted, and most aggregate is supplied from Cornwall, Leicestershire, and Wales. But the largest of these quarries, said Dr Gribble, can only produce about 2 million tonnes a year, and they do not have the reserves to allow for the increased output which will be necessary to meet the predicted growth in demand.

"We believe that Scottish quarries are in a position to supply a significant share of this demand. We also believe that we have the technology to supply that demand at competitive prices", said Dr Gribble and Mr Wilson. Their calculations depend upon the assumption, reasonably based on an extrapolation of present trends, that the cost of aggregate in 1980 will be about £10 for a delivered tonne at today's prices.

All that is required, they argue, is a novel modification of the present system of quarrying. They suggest that massive quarries, with reserves of over 100 million tonnes, should be developed in remote, granitic areas close to the coast. Each quarry would be worked to produce about 10 million tonnes a year. Such massive reserves, coupled with the intensive output, would reduce the cost of land fees, manpower, and plant renewal, and the consequent reduction of necessary capital investment would render the industry extremely cost-effective, it was claimed. And the location of the quarries near to the sea, preferably close by deep-water harbours, would provide easy access to a convenient and relatively economical means of bulk transportation. Labour requirements should provide little problem, according to Dr Gribble, as only about 100 men would be required to work a quarry of the envisaged size.

"Sites for these very large remote quarries would be carefully chosen and landscaped, screened from view, and so on," said Dr Gribble. He suggested that the development of 10 to 20 such quarries, located almost entirely on poor agricultural land, might be more desirable environmentally than the continual opening of smaller quarries in populated areas as the demand arises.

Mr Wilson's company already has two quarries on the east coast, at Peterhead and Inverkiething, which, it was claimed, could easily be developed on the proposed scale once the market became ready. And Mr Wilson knows of at least two other quarries in Scotland, which are in a similarly advantageous position.

The quarries at Peterhead and Inverkiething are, according to Mr Wilson, already capable of supplying material by sea not only to south-eastern England, but also to northern Europe where there is a similarly increasing shortage. He is quite confident that, if the suggested ideas are implemented, Scottish quarries could eventually provide as much material for export as for the home market; "we already have customers in Germany and France anxious to buy this material from us", he said. Both he and Dr Gribble are optimistic about the future of the Scottish quarrying industry which they believe could considerably bolster the future economy of Britain.

a department. A further problem is a shortage of reading matter. SISCON—the one-year-old Science in a Social Context project—is trying to fill this gap. A group of eight universities and one polytechnic running science studies or interdisciplinary courses have been given £50,000 by the Nuffield Foundation to produce course books which are flexible enough for a wide variety of courses. Dr W. F. Williams, the SISCON project coordinator, sees the job as essentially short term. The aim is to produce as wide a range of teaching material as possible and if within ten years this is not being supplemented and replaced by books written outside SISCON the whole idea will probably have been a failure. Dr Williams is also toying with the possibility of sending a researcher to Nigeria to gauge the need for similar publications in Africa.

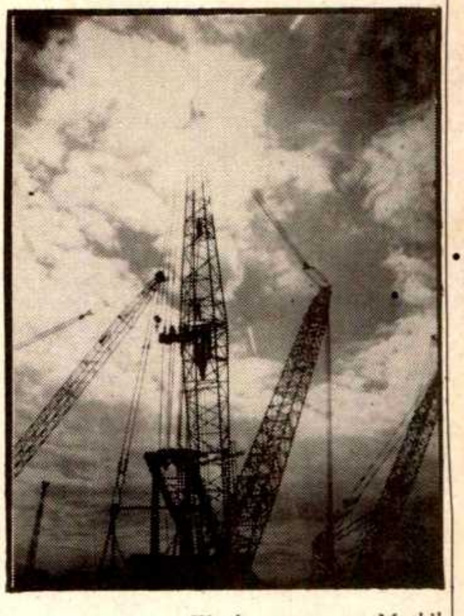
And what of the courses that do not attempt to break down the traditional boundaries? Dr Edge's institute in Edinburgh provides only one quarter to one half of a student's work for one year. Dr Edge aims to give students an awareness of science—its internal behaviour and its relations with the world—without taking up so much of his students' time to prevent them from progressing to scientific research if they wish.

Professor Jevons's department in Manchester similarly offers a single unadulterated science alongside science studies. But the entire course—science and science studies—is within a single department and Professor Jevons does not aim to produce scientists ready to do research. The students only spend half their time doing science and the other half looking at science in its social, historical and economic context.

Undergraduates prefer to be taught distinct subjects rather than a homogenised baby food, said Professor Jevons. It would be a pity to break down the real outlines of the traditional subjects in order to erect the flimsy bridges between the interdisciplinary courses. Yet students want to participate and debate and learn to take decisions. Since the well-worn precepts of the physical sciences are patently unsuitable material for this style of teaching, the best solution is to teach students something else besides their science. In Professor Jevons's department this something else is science studies, which can be taught by science teachers. This part of the curriculum develops the student's critical faculties which are by necessity blunted in the acceptance of scientific truths. The student becomes more aware of the role of science in the world and perhaps even a better scientist.

A final word of warning against the excesses of interdisciplinarity. Professor Kendrew reminded the meeting that certain "hard" subjects such as mathematics had to be learnt young or not at all. The early molecular biologists showed that it was a positive advantage to enter a field of which you had no previous knowledge, as long as the basic mathematical ability was there. And certain subjects could very properly be picked up by the student or graduate when he felt a need for them—witness Professor Kendrew's success with crystallography. ☐

As a rest from the round of lectures and seminars, British Association members had the opportunity to look around Redpath Dorman Long (North Sea) on the Fife coast. The little village of Methil on the coal coast of Fife once boasted a thriving colliery but since the 1960s unemployment had increased and the area had begun to show the inevitable signs of industrial dereliction.



Work returns to Methil

But in 1972, RDL (North Sea) set up at Methil, on the disused 136-acre colliery site, when the company was formed from a consortium of British and Italian interests. RDL, a subsidiary of British Steel, hold a 55% increase and the remaining 45% is held by three Italian companies with long experience in the design and construction of oil production platforms in the Mediterranean and the Middle East.

RDL's first steel jacket, for the Auk field, was successfully placed in position on its 280 feet deep water site this summer, and RDL are now building an even bigger structure for the 480-foot deep Brent field. As the weight of the structure varies with the square of the depth of the water, this platform is twice as high and four times as heavy as the 4,000 ton Auk platform. RDL have also started work on a comparatively tiny gas production platform for the Danish Dan field.

The site at Methil could certainly cope with at least one medium and one large-sized jacket at the same time, said a company spokesman. In this RDL think they have an advantage over some of the other steel platform constructors, who use the flotation tank method for building and floating out platforms and thus can build only one at a time. RDL build their platforms on dry land and then manoeuvre them onto submersible barges which carry them out to the site.

The Methil site has only 30 feet of water for floating out but this is perfectly adequate for the steel jackets which are towed out on their sides. RDL also have on offer a steel/concrete hybrid gravity platform but as yet have no takers. This design can also be built at the Methil yard and is suitable for water depths up to 600 feet, rivalling the concrete platforms.

Social concern

Mrs Shirley Williams, Secretary of State for Consumer Affairs, drew the attention of a British Association symposium to the difficulties that politicians find in keeping in touch with science. "When I was a minister with responsibility for science", she said, "I found that ministers with such responsibilities are not in touch with scientists" Speaking during a symposium on the British Association report on Social Concern and the Biological Sciences, Mrs Williams, a member of the working party who conceived the report, said that she had been dependent on a few and sometimes only one scientific adviser. There must be more round-table discussions such as the British Association can provide on controversial biological and medical advances, she said. Parliament badly need greater education and more information. Many of these topics are very suitable one for a free non-party vote but only amongst informed men and women, Mrs Williams concluded.

The working party has been criticised in several quarters for producing a non-report. Countering such criticisms, Professor Bodmer promised that they would now draft concrete proposals and press for these to be put into effect. The working party's life is to be prolonged by a fresh infusion of funds from the Leverhulme Trust, and in its future incarnations will widen its scope to cover other biological advances such as the genetic manipulation of bacteria, but would not venture into thorny paths such as pollution, population and behavioural control. ☐

Science and politics of molecular biology

by Eleanor Lawrence

MORE money for molecular biology could be the most cost-effective way of reducing Britain's massive health bill, according to Professor John Paul of the Beatson Institute for Cancer Research. Professor Paul told the British Association Annual Meeting that Britain is now approaching the limits of what our society can pay for in terms of direct medical care, and the only way to eventually reduce the bill was by a three to four-fold increase in government funding for biomedical research and especially for molecular biology.

Ill health costs Britain about one quarter of the annual Gross National Product each year when loss of production and social security benefits are included. But medical research accounted for £800 million, only 0.15% of the total GNP (1972 figures), of which the government contributed £25 million to basic research through the Medical Research Council. Defence research on the other hand was supported to the tune of £250 million in the same year.

In the past, biomedical research had brought the infectious diseases more or less under control, said Professor Paul, and indeed we could not afford such massive spending on ill health now, if this had not happened. But we are now left with a residue of degenerative diseases and the second most frequent cause of death—cancer, which had proved difficult to combat by traditional medical means. Professor Paul believes that we are on the brink of a rapid advance in the understanding and treatment of cancer, and that this will result from the application of the ideas and methods of molecular biology.

Twenty-one years of basic research in molecular biology have brought it to a point at which, through the relatively new branch of 'molecular pathology', its principles and methodology can be applied to the study of disease. About £1 million is spent on molecular biology, of which the MRC contributes the greatest part. Although research into molecular biology takes up about 5% of the MRC budget, in national terms this is very modest financing compared with for instance the United States. Professor Paul believes that molecular biologists should not hesitate to become involved in the 'political' arena and argue on political and social grounds for an increase in government finance as it is becoming increasingly clear that molecular biology ("that apparently most abstract of biological sciences") is a highly political subject. □

Room at the top in space

A YEAR ago the ministers of the 10 European Space Research Organisation (ESRO) countries reached final agreement on the formation of a single European space body—the European Space Agency, and a package of projects on which to embark itself. The Agency was due to come into force on April 1 1974, already three months later than the original target date, but in the event this date too proved ill-chosen. The ESA is still not in being.

The lapse is more apparent than real. The money is secure with guarantees from each of the 10 member countries and with what amounts to a bond deposited on the largest project of all—the $300 million development of the Spacelab element in the American space shuttle programme for the 1980s.

Most of the planned projects are trundling on and fairly well on schedule and within their budgets. A key meeting of national delegates takes place on September 27, and at the full ESRO Council on October 8 there is every hope that 'baptism' of the new management can be confirmed.

It may take another two years for the ESA Convention to be ratified by all 10 member states. In the meantime the organisation continues to run on the ESRO Convention. ESRO is already a very different animal from the ESRO of last year. The total budget this year is $240 million, against a mere $40 million in 1973. The Spacelab contract has been let. The more than two years of stalemate on the joint US-ESRO-Canada Aerosat project—to set up a pre-operational system for evaluation of air traffic control and navigation satellites in cooperation with air-lines flying the Atlantic—has been resolved. This has hinged on what to Europeans has seemed a technicality, finding an acceptable industrial partner among American companies to act for the US Government opposite ESRO and Canada on the management board. The Memorandum of Understanding between the three parties originally to be signed in January 1972 was finally completed in Paris last month. Last week the Aerosat Board chose Comsat (out of five contenders) as the American partner and its head visits Paris soon to persue details. The target date for launching the first Aerosat over the Atlantic, mid-1978, should now be met.

The credit for most of this must go to the head of Administration, Roy Gibson (pictured above), who has been acting director-general for the past months and was ESRO's chief negotiator at the original Aerosat talks in 1971. Latterly the work of the eight senior posts in ESRO has been shared between three people, with Gibson sustaining the lion's share.

In the face of the increased volume of work this hiatus at the top at ESRO has been serious. Two of the best men among ESRO's senior and most experienced staff have left for good. The five senior posts that are vacant cannot be filled till the director-general is appointed, since political considerations enter into the distribution of seats among the member countries. Of the sitting executives, one is English, one a Swede, one Italian. The most widely promoted candidates for the top job are the French ex-head of ELDO, the much respected General Aubinière, and a German civil servant, Dr Schmidt-Kuster. There is also a case to be made for Gibson himself. □

Plugging the hole in Skynet

from Angela Croome

"TRYING to pour a water-butt through a bootlace" was how one Services officer described pre-satellite long-distance communications for defence. That was in 1969 when Britain first adopted a satellite-borne defence communications system—*Skynet*—for its own exclusive and interference-free use, making Hong Kong and ships at sea (in the Gulf of Carpentaria for example) as instantly accessible as Aldershot from Whitehall. Although the satellite itself, Skynet I, was built (to a tight £6 million contract) by Philco-Ford in America, the hard-headed specification was British and the ground-based terminals to communicate with it were British-designed and built. The Skynet system must rank as the most enterprising and successful British space venture yet achieved with several world 'firsts' to its credit. It was the first operational defence system to use a geostationary satellite; and, the 3½-foot shipboard terminal, Scot, remains the smallest and most ingenious 'earth-station' for satellite communications yet developed, and the envy of the US Navy.

The original Skynet satellite (Skynet I) has now exceeded its planned operational life and is due for replacement. To ensure continuous operation in all circumstances the original satellite, poised over the Indian Ocean, was duplicated. The second could act as standby if the first developed faults; otherwise it could provide additional capacity in an emergency. In the event only one of the Skynet I series ever became operational. In its companion the solid fuel packing of the centrally situated apogee motor had an undetected crack which caused the motor to explode on ignition, effectively disposing of the satellite at the same time. It seems that the single Skynet I in orbit managed to sustain its role without a standby.

Skynet: the ship-borne end

Last January the first of two replacements was launched by Thor-Delta from Cape Canaveral. It too came to grief so now there is the prospect of the second, Skynet II, phase of the system, also operating with a single satellite despite 100% planned redundancy. On this occasion the second stage of the launch rocket misfunctioned in the sense that the jets of the motor jammed hard over while firing, causing the vehicle to corkscrew wildly and subjecting the satellite to forces greatly exceeding those allowed for in the design specification. The satellite was 'missing presumed lost' for three days but was then picked up by a United States Air Force tracking station over the Pacific. It was still functional and responding to interrogation and commands but in a hopelessly low orbit. An attempt to 'kick' the satellite out to an orbit clear of the Earth's atmosphere with the onboard apogee motor failed. Nonetheless the British contractors, Marconi Space and Defence Systems Ltd, were well pleased with how well the spacecraft had stood up to its very rough passage.

In the last week of August the standby Skynet II was dispatched to the United States in readiness for launch in November to take over Britain's overseas defence communications role for a further three years. The overall design and engineering concept of Skynet II is similar to Skynet I; it may be considered a 'stretched' Skynet I. It is about a third larger than its predecessor, however, and has a considerably greater communications capacity. The ease, convenience and immediacy of 'voice-contact-by-satellite' has, not surprisingly, proved popular with the Ministry of Defence, as transatlantic businessmen similarly welcomed the coming of Intelsat.

Demand for faster telecommunications grows with supply. This has become a truism in the world of commercial telecommunications. Predictions of the growth of phone traffic and especially overseas phone traffic are always too low. No doubt the same can be said of defence communications. It is not difficult to imagine the asset that Skynet must have been and still is to the British bases in Cyprus during the recent emergency. Yet the future of Skynet is hazy. No decision has yet been made on a backup for Skynet II, although the end of its planned life is not so far off. Defence planning—where a new weapons system for instance takes ten to twenty years to develop—has long made nonsense of five-yearly changes in Government. Can anything so fundamental to the total defence operation as communications, be left to the shifts of party politics? While Britain continues to have overseas defence commitments, a return to "bootlace" communications would be a false economy.

Life sentence

AN astronomical surprise for dilettantes and professionals alike was provided by Sir Bernard Lovell during a discussion at the BA meeting. He delivered a severe body blow to the popular notion that there may be around 100,000 technological civilisations in our Galaxy.

In the past, Sir Bernard said, one extremely important factor had been omitted from the calculations. Nobody had yet taken account of the fact that at least 90% of the 100,000 million stars in the Galaxy are K or M stars, which exhibit characteristics that could well preclude the development of organic life in their vicinity. For a start, they only exist for about 10 to 100 million years before becoming black dwarf stars.

It is extremely unlikely that these stars could develop a solar system in so short a time, and in any case that hardly seems long enough to allow for the evolution of an advanced civilisation. Which is just as well really, because any organic life which might evolve would find itself in an extremely inhospitable environment.

Sir Bernard explained that K and M stars have extraordinary flare properties: blasts often last for periods of up to several hours, during which the normal intensity of emission doubles. The radiation effects would of course be quite lethal to most complicated life forms. □

● BECAUSE of an error in transmission, *Nature's* account of the recent NAS statement on plasmid engineering contained an inaccuracy which might have caused a misunderstanding about the moratorium. Under the heading of types of research which should be halted, we listed "autonomously replicating bacterial plasmids that might result in the introduction of genetic determinants for antibiotic resistance or bacterial toxin formation into bacterial strains that do not presently carry such determinants . . ." This should have read: "Construction of new autonomously replicating bacterial plasmids . . ." etc. We regret any inconvenience this error might have caused. □

Canada gets a new science minister

from David Spurgeon, Ottawa

'Bud' Drury: experienced Trudeau man

CANADIAN scientists are pondering the implications of federal government appointments that have followed in the wake of July's general election. A Cabinet shuffle has brought a new minister and secretary (deputy minister) to the Ministry of State for Science and Technology, which celebrates its third birthday this autumn.

Actually, "new" is not quite the word for the man named to head the ministry as successor to the Hon. Jeanne Sauvé, now minister of Environment Canada. C. M. ('Bud') Drury is not only one of the most experienced members of the Trudeau Cabinet, having served for some years as president of the Treasury Board—he was also *de facto* minister of science before such a post existed officially. This responsibility fell to him for a number of reasons: his post with the Treasury Board, his position as the minister to whom the National Research Council of Canada reported, and his chairmanship of the Privy Council committee on scientific and industrial research.

In addition to the science and technology portfolio, Mr Drury has been given Public Works, and the question scientists are asking is, does his dual appointment indicate a downgrading of science in the priorities of the government, or does this appointment of a senior and experienced Cabinet minister indicate an upgrading of the ministry?

In terms of experience, Mrs Sauvé was a junior minister, science and technology being her first ministry appointment. Her move to Environment Canada can only be seen as a promotion, because it is a far larger department.

Her predecessor, Alastair Gillespie, who was Canada's first science minister, was also a new boy, who has since been promoted to the Ministry of Trade and Commerce. The fate of these first two science ministers confirms the idea that the government has been using the post as a training ground for rookies.

But now the issue is complicated by the rumour that Mr Drury's appointment as science minister is a temporary one. This poses two questions: Who will be the permanent minister? And will the new appointee have more or less political clout than Mr Drury?

Some see Mr Drury's appointment as a holding operation—yet a choice that will assure no reduction of importance accorded science in federal policy, because of his personal experience in the field. Because the ministry is not an operational department, its political influence depends largely on the status of the minister, or the importance attached to the portfolio by the Prime Minister, or both.

The implications of the new deputy minister's appointment are not entirely clear, either. Formerly deputy minister of health, Dr Maurice LeClair is expected by some to exert a more cohesive force on the day-to-day operations of the science ministry than his predecessor, Dr Aurèle Beaulnes.

But some expect his policies to be a good deal less open: Beaulnes was always ready to talk to the press (usually at great length); health ministry officials have been more secretive and restrictive. In fact, Dr LeClair went so far last spring as to issue a directive prohibiting health department scientists from attending a forum on science policy set up by SCITEC, an umbrella organisation that is striving to represent all branches of science and technology in Canada. The given reason was "that this whole area of science policy is at such a ferment at the present and so deeply tied in with basic government policy that it would be inappropriate for our scientists to be in attendance at the SCITEC meeting."

Later, on the day of the conference, after a public service union pointed out to the department that the directive abrogated personal freedom, and that a scientist was already bound by an oath of confidence to the government, the department modified its stand: its scientists could attend as long as they did so as individuals and not as representatives of the department.

Both Drs LeClair and Beaulnes have medical backgrounds, which is a cause for concern among some scientists who feel this key post should be assigned to someone whose training and experience better equips him to understand research in all fields.

First Netherlands satellite in orbit

THE first Netherlands scientific satellite ANS was launched from the Western Test Range in California by NASA on August 30, 1974, by a four stage solid fuel Scout rocket serving as launch vehicle.

The Astronomical Netherlands Satellite carries three scientific experiments for astrophysical observations and comprises instruments for the measurements and classification of stellar UV radiation and X-ray sources in space.

The spacecraft's systems are in good condition and the attitude acquisition phase during which one side of the satellite will be locked to the sun, is running as planned. However the rotation around the Earth differs from the original planned near-circular orbit of about 500 km altitude as present orbit has an apogee of 1150 km and a perigee of 260 km.

In the coming days all systems of the satellite will be checked and calibrated before measurements are initiated. In spite of the deviation in altitude it is expected that by reallocating and adapting the observation programme the planned investigations can be executed according to schedule and will last for at least half-a-year before leaving the sun-lit area. For the first time complete operation control of an European satellite is performed at ESOC, Darmstadt, in Germany, by a team consisting of Dutch NLR and ICANS members.

Indians' blast

A PUBLIC opinion poll of 250 adult literates in each of the cities Bombay, Calcutta, Delhi and Madras shows how strongly the Indian public feel about the recent nuclear blast. The questionnaire conducted by the Indian Institute of Public Opinion reveals that 89% of the sample believe that the test has raised India's prestige in the international community and 80% believe that peaceful nuclear explosions will help faster economic growth through use in mining and other fields. Nearly a half want to supplement the peaceful uses programme with a programme to build nuclear weapons while only a third of those questioned wanted the government to use nuclear energy solely for peaceful purposes.

news and views

Successor to Anger's gamma camera?

DURING the past fifteen years gamma cameras have been introduced into the regular hospital armoury of nuclear medicine In the hands of a specialist this camera became a diagnostic tool of great importance, particularly for detecting and localisating cancer lesions The generation of γ cameras in current use derives its pedigree from the concept of Hal Anger (*Rev scient Instrum*, **29**, 27, 1956), who used lead collimators to produce an image of the radioisotope distribution in a scintillating crystal viewed by an array of photomultipliers On page 132 of this issue of *Nature,* Todd, Nightingale and Everett propose a new principle of γ-ray imaging Using arrays of position-sensitive detectors and selecting multiple interactions it becomes possible to calculate the position of the source of a γ ray from the formula for Compton scattering In this way the use of collimators is completely avoided The image formation in the proposed system is different from that in the Anger type of camera Instead of building up the image from a dot pattern, the Compton γ camera produces families of ellipses with the common point corresponding to the source of radiation

The method of implementation of the new concept is hardly mentioned in the communication In fact, the success of the proposed system probably depends in a most crucial way on the nature of the radiation detectors and on efficient, very fast collection of data Recent developments in the technology of solid state detectors and the availability of very fast mini-computers are making the proposals a practical possibility, but it is worth noting that to date no commercial γ cameras based on semiconductor detector technology are offered The Southampton group is therefore attempting to improve the design of the gamma camera in more ways than one

The main advantages of the proposed new system lie, first, in its ability to collect γ radiation over a larger solid angle than is possible through a system of collimating holes, with a corresponding reduction of the necessary patient dose, and, second, in the possibility of attaining higher positional resolution than the existing cameras Modern high performance Anger cameras have resolution indices of about half a centimetre The authors' estimation of the resolution possible in their system is in the region of 1–2 mm The ability of detecting smaller malignant lesions is always uppermost in the minds of clinical diagnosticians

With a suitable fast data collecting system the high potential sensitivity of the Compton γ camera will make it the instrument of choice for fast dynamic studies with radioisotopes, for example, for the study of cardiac output and the blood circulation in limbs Furthermore, as Todd *et al* indicate, the computing system associated with the camera can select events originating at a certain depth in the body and can thus yield the tumographic information in any desired place

It remains to be seen if the Southampton group, after presenting a new and exciting idea, will be able to break through the technological barrier and produce a working model of the camera that will make it possible to evaluate all the predictions and expectations of its performance

K V ETTINGER
D A CAUSER

What makes a molecule odorous?

THE olfactory quality of a molecule has always proved notoriously hard to predict, but another attempt has recently appeared (Schiffman, *Science,* **185**, 112, 1974) This work is mathematical in nature and applies multidimensional scaling to known data

How much progress has been made since the confrontation between Amoore and Wright in the pages of *Nature* a few years ago (**233**, 231, 270, 1971 and **239**, 226, 1972)? In particular, can one yet distinguish between the theories of Amoore, Wright and Davies, preferably by a clear refutation of two of these theories?

The answers to these questions are complicated by the modifications that all three theories have undergone in recent years, and it seems also that the number of olfactory theories is growing, rather than diminishing (A full account of the principal theories and results up to 1970 may be found in *Handbook of Sensory Physiology,* **4**, (Part 1, Olfaction), Springer-Verlag, 1971)

Schiffman uses measures of psychological similarity as the input for her multidimensional scaling if two substances are judged by human subjects to have quite similar odour qualities, these two substances are placed near each other in multidimensional quality space Of course this is not new in itself, but what is new is that the known similarity data are now re-analysed by a general non-metric multidimensional technique, which shows that only two dimensions are required to account for the known similarity relationships between many different compounds

What new information emerges from such two-dimensional odour maps? First, it seems that there are no sharply defined areas which would correspond to possible 'primary odours'—the odour types all merge into one another, for example, from floral and fruity areas to areas of resinous (for example, turpentine-like) odours Or, in Schiffman's words "There are no clear psychological groups or classes of stimuli, merely trends" Unfortunately, she does not present any data on 'musky' compounds, usually regarded as constituting a well-defined group

Second, can these psychological similarities be related to the usual molecular properties? Schiffman claims that Amoore's original (1952) theory that molecular shape

This is the last News and Views prepared by Sally Bunney who after nearly seven years with *Nature* is off to a post as medical librarian in Oman She goes with the best wishes and affection not only of the staff but also of an army of correspondents Mrs Gillian Boucher takes over Miss Bunney's position uniquely determines olfactory quality is not confirmed, but this conclusion was reached some years ago by Amoore himself, who modified his "site" theory to account for trends rather than for sharp "fit" or "not-fit" categories of molecular shapes.

As regards Wright's spectral theory, this gives a good correlation with the odorants in two broad categories (pleasant and unpleasant), but five pleasant odorants are incorrectly classified as unpleasant on the basis of their Raman spectra.

As regards the "penetration and puncturing" theory of Davies, in which the lipid part of the plasma membrane is held to be responsible for olfactory effects, Schiffman reports that lipid solubility may be necessary for olfactory stimulation to occur (since all the odorants were soluble in ether and there was no trend of odour type as a function of water solubility). There is apparently no evidence against the two-dimensional odour map earlier published by Davies.

So no single physico-chemical variable can account for odour qualities. But how many such variables are required? Schiffman used suitability weighted functions of Raman intensity, molecular weight, number of double bonds, functional group and cyclic structure, these being taken as simultaneous but separate variables which, empirically weighted in combination, are used to correlate molecular properties with the psychological similarity map. Altogether 25 weighting factors are chosen to give the best fit. The final correlation coefficient is 0.76, which in these circumstances is distinctly disappointing. as Wright has pointed out, Amoore's odour similarity values (admittedly over a limited range of odour) have a correlation coefficient of 0.85 with the square root of the molecular weight. Furthermore, as Mazziotti has shown (*Nature*, **250**, 645, 1974), there is even quite a fair correlation between odour type and boiling points, particularly within groups of substances whose molecules have the same functional group. Perhaps Schiffman's most important contribution to the debate is that molecular shape as such is not said to be significant.

Future progress in the field of odour must surely be concerned with mechanism of the initiation of the nervous impulses in the olfactory epithelium. Adrian, many years ago, found that different regions of this epithelium in the rabbit and the hedgehog are relatively selective to stimulation by certain types of odorant molecules. Since the lipids may well be responsible for the initial adsorption of the odorants from the air stream, and since the lipid structure may then be locally disorganised by the odorant molecules, one predicts differing properties of the lipids in the various regions of the olfactory epithelium.

This urgently requires testing experimentally by an analysis of lipids taken from the various regions of the epithelium. Only by experimental tests of such physical theories is there likely to be real progress in the understanding of olfaction.

J T DAVIES

Clonal selection hypothesis under fire

BIOLOGICAL research in recent years has acquired various dogmas, the most famous of which is probably the central dogma of the molecular biologists. Immunologists, not to be outdone, have espoused the clonal selection hypothesis as their central concept to provide a framework for the understanding of classical antibody synthesis. A recent statement of this notion says "Antibody molecules consist of heavy and light polypeptide chains, the variable sequences of the 110 N-Terminal amino acid residues of these chains determine antibody specificities; lymphocytes are cells that express one pair of structural genes for these sequences; *one lymphocyte and all of its descendants make identical antibody molecules* (my italics); these are placed as receptors for antigen on the outer lymphocyte membrane; the attachment of antigen to the combining sites of these receptors is a step in lymphocyte stimulation leading to cell proliferation, antibody secretion, and thus to an amplification of this selected antibody" (second *Annual Report of the Basle Institute for Immunology*, page 1).

Although several of the items of this concept thus stated could be questioned, the present interest is in the italicised phrase which seems to be contradicted in part by a recent communication by Cunningham and Fordham (*Nature*, **250**, 669–671, 1974). These investigators present evidence that sometimes single antigen stimulated cells can engender clones of cells of which not all the members of the clone are producing the same kind of antibody.

Mouse spleen cells were cultured *in vitro* in the presence of bacterial lipopolysaccharide and sheep red blood cells. After 24 h the cells were harvested and dispersed in microdroplets under oil in fresh medium with a mixture of red cells from two standard sheep and complement. In these circumstances the antibody from some of the cells diffuse into the surrounding medium and, in the presence of complement, soon lyses the sheep red cells giving rise to a more or less clear plaque around the antibody-producing cell. The plaque-forming cells were isolated by micromanipulation from the microdroplets and seeded on to a feeder layer of irradiated mouse spleen cells and sheep red cells were again added. After about 2 days some of the isolated plaque-forming cells had divided. Their progeny cells were re-isolated where necessary in the presence of trypsin to disaggregate them and they were added individually to a mixture of fresh medium, complement and red cells from the two standard sheep. These mixtures were pipetted into Cunningham slide chambers (Cunningham and Szenberg, *Immunology*, **14**, 599, 1968), and the resulting (19S) plaques were characterised as either clear, partial or 'sombrero'. Clear plaques are supposed by the authors to arise around cells which have produced an antibody (or antibodies) which recognises antigens common to both the target red cells. Partial plaques have some unlysed red cells and (despite the lack of a morphological criterion of distinction) these are interpreted as arising around cells which produce antibody capable of binding effectively to only one of the kinds of red cell. Sombreros are supposed to arise around cells which are producing antibodies for common specificities but with higher avidity for one of the kinds of red cell than the other. About 10% of all isolated cells formed clones and of these about 10% were found to give rise to heterogeneous plaques. Most of the heterogeneous clones had only two kinds of plaque forming cell. Two had three kinds of cell and one clone (of twelve cells) gave rise to four clear plaques, five sombreros and two partials. One could not be diagnosed.

On the basis of these results the authors postulate that, contrary to the precepts of the clonal selection theory, diversity in relation to antibody-producing capacity can commonly (if not always) arise after contact with antigen rather than be pre-existent. The approach is ingenious and owes much to the use of the polyacrylamide gel microculture plates developed by Marbrook and Haskill (*Cell Immunol*, in the press). Whether the evidence presented by Cunningham and Fordham will withstand the barrage of criticism which will be put up by the immunological establishment remains to be seen but it is healthy that after such a long run unopposed, the Clonal Selection Theory is taking a few knocks.

A J S DAVIES

Can neutron starlight be seen?

AMONG the more exotic inhabitants of our Galaxy are the enigmatic neutron stars Although these objects are about the same mass as ordinary stars, they are only a few kilometres in diameter, and are therefore unbelievably dense So much so that their constituent atoms are entirely crushed by gravity into neutrons Despite having been predicted theoretically for many years, firm evidence for their existence had to await the discovery and interpretation in the late 1960s of the now famous pulsars Pulsars are radio sources which emit bursts of energy at very regular durations, usually about once a second Current models of these cosmic clocks assume their regular ticking to be caused by the rapid rotation of an extremely compact object coupled to a magnetic field The compact object is generally thought by astronomers to be the long awaited neutron star, born during the violent aftermath of a supernova explosion

Models of the pulsar mechanism are necessarily tentative, but the pulses of radiation are believed to originate not from the neutron star itself, but rather from the surrounding magnetosphere This then leaves open the question of what radiation might be expected direct from the neutron star surface The answer to this depends on the temperature of the surface, which is really an unknown factor General considerations, however, point to this radiation being in the X-ray region, so that the exciting possibility emerges that the nascent science of X-ray astronomy might provide important data for astronomers studying neutron star structure and pulsar mechanisms

Actually, the idea of searching for X-rays from neutron stars is not new Before the appearance of pulsar astronomy, it remained the best hope for the eventual discovery of these strange objects The excitement generated during the past few years by the spectacular pulsars has, however, quite eclipsed the consideration of X-ray detection of neutron stars, and the subject makes a new appearance in the August 9 issue of *Science* (**185**, 487–490, 1974) The resurrection is promoted by two American astronomers, Greenstein of Amherst College Massachusetts, and McClintock of MIT These authors point out that this X radiation bears the same relation to neutron stars as continuum starlight does to ordinary stars And where would astronomy be without starlight?

Recently, strenuous efforts have been made to construct theoretical models of neutron stars The problems involved are formidable, for in almost every respect these objects represent matter in an extreme condition Indeed, the entire neutron star may be envisaged as a sort of gigantic atomic nucleus A correct understanding of such a strange object requires advances in several separate branches of physics Consequently, a direct observation of neutron star radiation would provide the only source of observational support for theories of matter in this high density condition

Specifically, Greenstein and McClintock suggest five pieces of information which could be deduced from a careful measurement of X-ray spectrum The spectrum itself would give the surface temperature of the star and hence, knowing the total flux, its radius could then be estimated, thereby confirming (or not) that the source really is a highly compact neutron star In turn the radius could be used to determine the mass and the pulsar magnetic field, at least approximately In addition, a knowledge of the magnetic field, temperature and mass enables the age of the neutron star to be calculated, with the possibility of a fruitful comparison with the associated pulsar age which may be deduced from the rate of slowing down of the pulsar period Finally, it is in principle possible to determine the internal temperature of the star, with a possible cross check here too from the details of the quasi-exponential period decay which has been observed to occur after peculiar period jumps in the radiation pulses

In their article, Greenstein and McClintock outline three theoretical procedures which may be used to obtain an estimate of the temperatures of neutron stars The first is simply to try to calculate the rate of cooling as from the time of formation The second is to examine various dissipative processes which generate heat inside the object as it rotates In both these methods the condition of the relevant theoretical models does not inspire much confidence in the result, so that a crude upper limit may be placed on the temperature by summing the total available energy Turning to the observational aspects of the subject, the authors paint a rather pessimistic picture There is no observational evidence at all yet for thermal X rays from neutron stars The best potential candidates are thought to be the middle aged pulsars—perhaps 100,000 years old —whose direct radiation would not be swamped by the pulsar radiation from the magnetosphere From the present non-detection of X rays from the known pulsars by the Uhuru X-ray observatory, upper limits on the temperature of 21 nearby pulsars are quoted A comparison of a sample of these limits with the theoretical values obtained using the three methods described above is presented Unfortunately, in all cases the theoretical values lie well below the observed upper limits, so that little of value may be gleaned from this

Despite the rather discouraging situation so far, Greenstein and McClintock argue their case well, and it would be surprising if further observational and theoretical work were not initiated on this difficult but important topic for astronomers And in view of the fact that the neutron star represents the most extreme density of matter in the observable Universe (greater densities lead to unobservable black hole matter) there is also a general scientific interest in their direct observation

P C W DAVIES

Checking on laser fusion

from a Correspondent

IN May this year, KMS Fusion Inc claimed to have produced, for the first time in the United States, thermonuclear neutrons from a laser-compressed deuterated target (The background to the laser-compression approach to fusion is outlined in *Nature*, **239**, 139 and 129, 1972) K M Siegel, chairman of the company, later amplified some of these claims, which are the outcome of a private corporate investment which now amounts to $17 5 million, in a talk given at Harwell on July 8

Between May 1 and July 8, about 35 neutron-producing shots on deuterated and D–T targets had been made, of which 80% were successful The prompt neutron yield (measured with a time resolution of 2 ns) was as high as 5×10^5 when the target chamber was evacuated, the total yield increased to $1\ 8\times10^7$ when deuterium gas was admitted to the target chamber (The latter result duplicated earlier Russian yields, in which neutron time-of-flight measurements were made with a detector resolution of 5 ns over flight paths of more than 3 m) X-ray pinhole photographs, with a spatial resolution of 5 μm, demonstrated that the implosion and compression of a hollow target was spherically symmetric Time-resolved X-ray emission measurements suggested that a density increase of 80 times occurred when two opposing Nd-laser beams (total energy $<$ 100 J) were focused by ellipsoidal mirrors on to the (suspended) solid state target

This compression, and the prompt neutron yield, were said to be consistent with computer predictions

These claims have unleashed some controversy within the international scientific community Everyone agrees that 'scientific break-even', in which the energy of the neutron output is roughly equal to that of the incident laser pulse, would produce so many neutrons (~ 10^{14}) that a detailed and unambiguous determination of their spectrum and physical origin would be possible Severe problems arise, however, with neutron diagnostics at the present level where, relatively speaking, only a handful of neutrons (10^{-8} of the break-even yield) are available for examination Additional diagnostic techniques which can provide independent checks on plasma density or temperature are therefore necessary until well documented 'break-even' yields have been achieved

At densities less than approximately 10^{19} electrons cm^{-3}, measurements of refraction or scattering have often proved a reliable diagnostic Attwood and Coleman (*Appl Phys Lett*, **24,** 409, 1974) have recently discussed a holographic micro-interferometer, operating at a wavelength of 5320 Å, which measures the optical path length through plasmas at densities exceeding 10^{20} electrons cm^{-3} The duration of their laser pulse 10^{-10} s limits the spatial resolution to 30 μm for plasmas moving with velocities less than 3×10^7 cm s^{-1} Shorter laser wavelengths permit penetration into denser plasmas, and reduce beam deviations caused by refraction at strong density gradients (Thus, the United Kingdom Culham Laboratory is using a 3471-Å holographic interferometer for density measurements with a resolution of 10^{-9} s on sub-kilojoule CO_2 laser-filling experiments) For penetration to densities of interest in the context of laser fusion (10^{25} ~ 10^{27} electrons cm^{-3}) sources of high spectral brightness with wavelengths of less than 10 Å will be needed, there is therefore a considerable incentive to develop coherent X-ray sources for refraction and absorption measurements in this regime

Temperature measurements are also difficult (see Puell *et al*, *Z Naturforsch*, **25a,** 1815, 1970) and at high incident intensities the electron velocity distribution may not be completely Maxwellian For example, at Los Alamos, McCall *et al* (*Appl Phys Lett*, **25,** 108, 1974) have demonstrated a significant departure from equilibrium when a thin polythene (slab) target is irradiated at incident intensities of 10^{16} W cm^{-2} ($\lambda = 1\ 06\ \mu m$) or 10^{14} W cm^{-2} ($\lambda = 10\ 6\ \mu m$) For these measurements a six-channel Bragg crystal spectrometer was used to analyse time-resolved X-ray bremsstrahlung between 4 and 50 KeV with an energy resolution of some 4–12% Conventional scintillator/photomultiplier detectors were used in this work, but for faster detection Key, working with the Garching group, has recently shown that sub-nanosecond X-ray vacuum photodiode detection should be possible Peacock and colleagues have exploited the rich line spectrum emitted from partially ionised $(CH_2)_n$ plasmas, a grazing incidence spectrograph, intensely calibrated over the range 5–45 Å (*J Phys E*, **6,** 854, 1973) is used to unfold line intensities and recombination continua Considerable care is required to achieve reproducible results with such photographic techniques

A wide range of experiments has been reported from laboratories in France, Germany, Japan and the Soviet Union, in which the intensity and spectrum of light back or side-scattered from the target at frequencies near the fundamental laser frequency and its harmonics have been measured, such data should provide valuable information concerning nonlinear absorption mechanisms which are of importance to the concept of laser fusion Within the next decade compression experiments capable of delivering some 1 to 10 kJ of laser energy on to carefully designed targets are planned at KMS, the University of Rochester, Lawrence Livermore Laboratory and Los Alamos in the United States, the Lebedeev Institute in the Soviet Union, and probably at other national laboratories as well The outcome of these experiments will be crucial, since the demonstration of a break-even yield of neutrons would constitute a definitive milestone in the history of thermonuclear research

A new role for the glial cell?

from a Correspondent

GLIAL cells are derived embryologically from the same group of ectodermal cells that gives rise to neurones and they were distinguished from nerve cells more than a century ago by histologists About half the volume of the vertebrate brain is composed of glial cells and these cells outnumber neurones by a factor of roughly 10 to 1 But although glial cells can be studied by morphologists, physiologists know very little about their function It is usually argued that because nerve cells are closely enveloped by glial cells the latter provide a 'supportive function' but just what kind of support has not been clear

A clue that one aspect of this support might be to provide a protein vital for the growth and differentiation of the neurone can be found in the discovery, reported by Longo and Penhoet (*Proc natn Acad Sci, USA*, **71,** 2347, 1974), that a tumour derived from glial cells contains the protein known as nerve growth factor (NGF) The protein was obtained from a rat glioma tumour by the method used to purify NGF from mouse salivary glands It cross reacted with antiserum to mouse NGF, gave similar responses to NGF in two biological assay systems and three of the proteins in the tumour extract had the same isoelectric points as the three fractions of mouse NGF The tumour extract, however, also contained other proteins, as was shown by electrophoresis and bioassay

Nerve growth factor is a basic protein (or class of proteins) which stimulates the growth of embryonic sensory ganglia and whose presence is required for the normal growth and differentiation of peripheral sympathetic noradrenergic neurones The fact that high concentrations of NGF are only found in the submaxillary gland of the mouse and, even there, only after puberty, has long been a puzzle Because animals are uniquely sensitive to the neutralisation of NGF by antiserum during the perinatal period, it has been argued that there must be an important unidentified source of NGF in the newborn animal Longo and Penhoet now suggest that this source may be in the glial cells they put forward the hypothesis that "glia synthesise and secrete NGF which then may act as a 'pleiotypic' stimulant for the neurone"

One of the properties of glial cells—their supportive role during regeneration of damaged nerve axons—is consistent with this hypothesis Noradrenergic neurones in the brain display a remarkable ability to regenerate after damage to their axons Studies in Sweden have shown that NGF injected into the cerebrospinal fluid or directly into the brain close to the locus coeruleus (the site of origin of a major group of central noradrenergic neurones) stimulates this regenerative process (Stenevi *et al*, *Brain Res*, **69,** 217, 1974) This team has also found that intracerebral administration to rats of antiserum to NGF inhibits the regenerative growth of central noradrenergic fibres (Bjerre *et al*, *Brain Res*, **74,** 1, 1974) Since it is believed that NGF does not readily enter the brain from the blood stream, the NGF required for the regenerative process must originate in the brain Could the ubiquitous glial cell be the source of NGF in the central nervous system?

It is to be hoped that the observations of Longo and Penhoet on the presence of NGF in a glioma will lead to attempts to demonstrate NGF in normal glial cells, possibly by the use of immunofluorescence histochemistry If these attempts are successful and if it is possible to demonstrate secretion of NGF from glia, then the glial cells will be recognised as a kind of endocrine tissue that is wrapped around its target cells, the neurones

Oppression or altruism in insect unions?

from our Insect Physiology Correspondent

THERE are differences of opinion among students of bee societies as to the origin and evolution of the worker caste Since the views of Hamilton were put forward in the 1960s, most authors have emphasised the idea of 'kin selection' favouring altruistic social behaviour the workers (the haploid partheno-genetic daughters of the queen) can promote the persistence of genes like their own more effectively by helping their mother to produce more siblings than by leaving home and mating on their own account But some authors favour theories of individual selection Either selection of mothers which can control their offspring, keep them in the nest and suppress their reproductivity, or selection of individuals, which, by their social characteristics of mutual tolerance and continued residence in the nest can benefit both themselves and their mother

Michener and Brothers (*Proc natn Acad Sci USA*, **71**, 671, 1974, *J comp Physiol*, **90**, 129, 1974) have now approached this problem by studying a small eusocial halictid bee *Lasioglossum zephyrum* which burrows in earthen banks Closely related (within the same genus) to solitary species, this bee is about as low in social level as any known eusocial insect, the average membership of a colony is only 14 bees As in other eusocial Hymenoptera, the males play no part in colonial life A large number of experimental colonies, maintained by Lubbock's classic technique, in earth between glass plates, were closely observed and the behaviour of marked individuals was compared and quantified statistically The queens are indistinguishable externally, although they have the largest ovaries, this is only the extreme of a continuum of ovarian size among the workers, the queens are best identified by their behaviour If the queen is removed her place is quickly taken by one of the workers

In her behaviour the queen is characterised by maximum general activity and work on the burrows, a maximum tendency to 'nudge' and disturb other members of the colony and thus to suppress their ovaries, and a maximum tendency (after nudging a bee) to back down the burrow and draw the nudged bee down into the depths of the nest where it will become occupied in constructing and provisioning Queens were also observed to eat the occasional eggs laid by workers Conversely the queen shows minimum activity in pollen collecting, and in guarding the nest, as well as in being nudged, or being induced to follow a backing worker

All this looks far more like oppression of workers by the queen rather than simple altruism by the workers, it suggests that queens have evolved to control their workers rather than that workers have evolved as altruists to help their queens Michener is therefore led to believe that control or 'oppression' by the queen has been of fundamental importance in the most primitive eusocial forms But, as he points out, worker altruism is a necessary consequence of eusocial systems and altruistic behaviour may well be evident sometimes even in the absence of control by the queen

Lassa fever and public health

from Arie J Zuckerman

LASSA fever was first recognised in 1969 and four epidemics have occurred in West Africa since then (*Nature*, **241**, 91, 1973) The severity of the illness is emphasised by a high case fatality ratio of 36–67% among patients admitted to hospital, person-to-person transmission, particularly in the hospital environment, and a high risk of infection to medical personnel To date, 20 medical workers have contracted Lassa fever and nine have died (Monath, *WHO Chronicle*, **28**, 212, 1974) In Africa, the virus was introduced into hospitals by African patients admitted with febrile illnesses which often mimic typhoid fever, malaria or yellow fever Secondary infections occurred among hospital staff and patients, but it seems that although the primary index cases were highly infectious, the secondary cases, for reasons that are not clear, were not

Lassa virus is a member of the arenovirus genus (invariably misspelt arenavirus) The virus is related both morphologically and antigenically to lymphocytic choriomeningitis virus and the viruses of the Tacaribe complex (haemorrhagic fever viruses of South America) A number of the related arenoviruses have been isolated from wild rodents and bats, and although it was postulated that Lassa fever is also a zoonosis, confirmation was not obtained until last month by Monath and colleagues (*Science*, **185**, 263, 1974) During the investigation of the epidemic of Lassa fever in Sierra Leone in 1972 (*Morbidity and Mortality Weekly Report*, **21**, 386, 1972) 641 small vertebrates were trapped Samples of organs and tissues were frozen in liquid nitrogen and sent for study to the Maximum Security Laboratory, Center for Disease Control, Atlanta, Georgia, at present the only laboratory equipped to attempt isolation of this virus safely Lassa virus was isolated from a single murine species, the multimammate rat *Mastomys natalensis*, which is a common commensal rodent in West Africa adapted to life both within houses and in the fields It is of interest that rodents of other species commonly found in the area of the epidemic in Sierra Leone (*Mus musculus* and *Rattus rattus*) were not infected, and it seems that these species either resist infection or avoid contact with infected *Mastomys*

The mode of transmission of the virus from rodent to man or from man to man is not known yet Similarly, the pathogenesis of Lassa virus infection in its natural host remains unknown It is, however, reasonable to expect that perhaps like a number of other arenoviruses, which induce a chronic virus carrier state in their natural hosts, for example lymphocytic choriomeningitis, a persistent infection with Lassa virus develops and both horizontal and vertical transmissions occur Rodents may excrete the virus in urine and saliva and may thus contaminate food, water or the air Monath *et al* suggest that the low level of sanitation, the storage of grain and food within houses and the ease with which rodents infest mud and thatch houses enhance the contact between rodents and man The means by which the virus is spread from person to person is not clear Medical attendants or relatives providing direct personal care are most likely to contract the infection Accidental inoculation with a sharp instrument or contact with blood has accounted for a few cases (see *Nature*, **241**, 91, 1973) Lassa virus has been isolated from the pharynx and urine of patients so that indirect airborne spread of the virus as well as mechanical transmission are most likely

Serological and epidemiological investigations have shown that Lassa fever seems to be limited to West Africa, and there is evidence that the virus was present in the Republic of Guinea in 1952, within the last two years it reached Senegal Mild and perhaps subclinical infections are now recognised to be not uncommon outside the hospital environment In the absence of a vaccine, available methods of control of community outbreaks are rodent control and quarantine. It has been pointed out previously, however, that the epidemiological propensities of Lassa fever may be very wide as a

[illegible]
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result of fast air travel Indeed, a number of cases have been treated in a London hospital Monath (*WHO Chronicle*, **28**, 212, 1974) wrote that a programme of surveillance must be instituted both in West Africa and in the developed countries To this one should add the provision of hospital isolation and laboratory facilities in selected centres

Sicily as part of Africa

from Peter J Smith

ACCORDING to Barberi *et al* (*J geophys Res*, **78**, 5221, 1973), the southern Tyrrhenian Sea contains an island arc structure which includes a sediment filled trench along the Ionian coast of Calabria, a metamorphic belt stretching along Calabria into Sicily, a calc–alkaline volcanic arc in an advanced state of evolution (Eolian Islands) and a back-arc marginal basin (Tyrrhenian Abyssal Plain) In short, the Tyrrhenian Sea area is readily interpreted in the light of the destructive boundary between the African and European plates But where precisely does the plate boundary lie in this region? McKenzie (*Geophys J*, **30**, 109, 1972) and several other workers have proposed that the boundary line actually crosses northern Sicily, in which case most of Sicily will be a part of the African plate New evidence in favour of this view has now come from two quite different sources

Evidence adduced by Barberi *et al* (*Earth planet Sci Lett*, **22**, 123, 1974) comes from the distribution, age, nature and palaeomagnetism of volcanic rocks in the east of the island, although the volcanics are only one of five principal provinces in Sicily In the extreme south-east lies the Ragusa–Iblei carbonate platform which is not affected by Alpine folding and seems to be an extension of the Sahara platform In other words, the southernmost part of Sicily is apparently linked geologically with Africa—a view supported by the existence of 20 km-thick continental type crust to the south of the island Central and western Sicily comprise a Mio–Pliocene resedimentation basin, and in the north there is a series of flysch nappes ranging in age from Cretaceous to Miocene But the extreme north-east of the island is a crystalline massif forming the continuation of the Calabrian metamorphic belt In other words, the northernmost part of Sicily is apparently linked geologically with Europe The view that the Euro–African plate boundary crosses Sicily is thus supported in general terms by the geology

For a more quantitative picture of Sicily's behaviour, however, it is necessary to look at the eastern part, which has been a site of dominantly basaltic volcanism since the middle Triassic (although only Cretaceous and younger rocks are exposed) The most recent episodes of (subaerial) volcanism are represented by Mt Etna and Mt Iblei, but all volcanic activity before this was submarine, giving rise to pillow lavas and hyaloclastics emitted through fissures in a carbonate platform The oldest rocks examined palaeomagnetically by Barberi and his colleagues were Upper Cretaceous dykes (with potassium-argon ages in the range 71–84 million years) cutting hyaloclastics of three different Cretaceous outcrops From Cretaceous to Upper Miocene no basaltic volcanism occurred in the region, although important activity has occurred since, principally in the vicinity of Iblei and Etna The second set of rocks studied was therefore of Iblei volcanics within the age range 1 7–5 4 million years

The palaeomagnetic results are simply stated The Upper Cretaceous pole is consistent with that for Africa obtained by Shazly and Krs (*Geol Rdsch*, **62**, 212, 1973) but significantly different from the mean Cretaceous pole for Europe The Plio–Pleistocene pole for Sicily is consistent with the mean Upper Tertiary–Quaternary poles for the Central European-Russian platform and for the French Massif Central lavas and with the Quaternary poles for Africa and Spain (Gerona volcanics) It thus seems that there has been no significant motion of eastern Sicily with respect to Africa since the Cretaceous On the other hand, Sicily has moved with respect to Europe since the Cretaceous, but not since the end of the Miocene The implication, therefore, is that (with the probable exception of the extreme northeast of the island) Sicily has been part of the African plate since the Cretaceous—and thus in all likelihood since at least the Triassic

Barberi and his colleagues then visualise Sicily's fitting into the evolutionary pattern of the region in the following way From the Upper Triassic to the Upper Cretaceous the African plate moved south-easterly with a sinistral shear movement with respect to Europe, resulting in a separation of the African and European plates and the formation of oceanic crust The northern edge of the African plate (and thus Sicily) then underwent distension—a process consistent with the alkalic products of volcanism observed in Sicily for the relevant period From the Upper Cretaceous to the present the motion of Africa with respect to Europe has been mainly compressional with minor dextral movement—a change also reflected in Sicilian volcanism Basaltic distensive volcanism was absent from eastern Sicily from the Upper Cretaceous to the Upper Miocene The occurrence of Upper Oligocene–Lower Miocene andesitic volcanism suggests, however, that the compression between Africa and Europe was accompanied by subduction of the oceanic crust previously formed between Sicily and Europe, a process which gave rise to island arc type volcanism towards the end of the Oligocene Crustal consumption lasted until the Lower Miocene, when the continental collision occurred, and since the late Miocene Sicily has been joined to Europe, as the Iblei palaeomagnetic data indicate

An obvious problem with this scheme is the post Miocene and current basaltic distensive volcanism in eastern Sicily, which is difficult to explain in terms of the general tectonic framework of compression The explanation put forward by Barberi *et al* is that continental collision has occurred at different points along the Sicilian-Calabrian segment of the plate boundary at different times, possibly because of the irregular shapes of the encroaching continents For example, the subduction of oceanic crust beneath the Calabrian arc is still active (hence the Eolian island arc), although the continental nature of the crust on both sides of the boundary suggests that crustal consumption is now nearly complete In any event, since the Lower Miocene eastern Sicily has been the border zone between colliding continental blocks and the oceanic lithosphere subducting beneath the Calabrian arc, and has thus probably been affected by local distension, notwithstanding the general overall compression

The second piece of evidence supporting the view of Sicily as part of Africa comes from laser geodimeter measurements between the island and Calabria on the Italian mainland Caputo *et al* (*Geophys J*, **38**, 1, 1974) report six sets of such measurements made between September 1970 and September 1973 Needless to say, even with lasers it is a difficult task to determine the very small distance changes likely to be involved over such a short period, and the variations in the lengths of the sides of the Sicily-Calabria geometric net are indeed found to be generally smaller than the measurement errors and data corrections Nevertheless, Caputo and his colleagues tentatively conclude that a statistically significant displacement between Sicily and Calabria took place between September 1970 and June 1971 During this period (but not subsequently) Sicily was displaced roughly northward with respect to the mainland, implying that it participates in the movements of Africa rather than Europe

Harmony of the spheres

from Robert W Cahn

VERY gingerly, the proponents of fibre reinforcement are beginning to approach the serious possibility of using fibre-reinforced materials for service at high temperatures—for instance, in gas turbine components One central difficulty about this is that the metallic matrix may chemically attack the fibres, for example, tungsten fibres interact chemically in a complicated way with a nickel matrix The other tricky problem is to keep the shapes of the fibres inviolate It is not too difficult to generate a regularly spaced array of long, thin fibres in a suitable matrix, much research has been done on the directional growth of monocrystals of appropriate binary and ternary alloys, consisting of aligned fibres (or plates) in a matrix The trouble is that a long thin cylindrical fibre is not in geometrical equilibrium with its matrix

In a slowly grown eutectic, the total quantity of fibrous phase will be in equilibrium with the total quantity of matrix the disequilibrium is purely a matter of geometry This has long been known to metallurgists familiar with ordinary mild steel The pearlite ($Fe+Fe_3C$) eutectoid lamellae, if sufficiently heated, turn into arrays of fine Fe_3C spheres The process, spheroidisation, is driven purely by the tendency to minimise the total area of Fe/Fe_3C interface The same can happen to a long fibre in a matrix

As so often in physical science, one of the key ideas comes from Lord Rayleigh In 1878 he showed that a cylindrical jet of water tends to instability it is apt to pinch off at intervals of about eight diameters Recently, McLean of the National Physical Laboratory, showed (*Phil Mag*, **27**, 1253, 1973) that, correspondingly, cylindrical inclusions of lead in aluminium split into separate short cylinders if the aspect ratio (length/diameter) exceeds 8 Each cylinder then gathers up into a sphere The relevance to the stability of a population of fibres is manifest Although aluminium–lead is no candidate for high temperature use, it is a useful model system

With the collaboration of Loveday, McLean (*J Mater Sci*, **9**, 1104, 1974) has now taken his investigation of Al/Pb alloys a long step forward The main improvement is one of technique instead of using X-ray microradiography between anneals, the investigators examined their foils in a 1-MeV electron microscope fitted with a heating stage With the aid of cinematography, and exploiting the high penetrating power of the energetic electron beam, the investigators were able to trace in detail the stages of spheroidisation and make accurate kinetic measurements In addition to the advantages of *in situ* micrography, they also achieved a much higher resolution by this technique

Correction

In the News and Views article "Bugs are Beautiful" (**250**, 533, 1974), the abbreviation AISB stands for Artificial Intelligence and Stimulation of Behaviour Group, the published explanation is incorrect

The lead inclusions in the Al+5wt% Pb alloy were turned into cylinders by mechanical swaging (there is no suitable eutectic) and foils cut from this material were heated in the microscope to above the melting temperature of the lead The spheroidisation process is governed by diffusion of matrix atoms through the cylinders, since the lead was molten diffusion was rapid and so was the spheroidisation The cylinders either broke up into shorter ones which then turned into spheres or short cylinders spheroidised directly, Rayleigh's criterion was approximately obeyed The detailed observations allowed the diffusivity of aluminium in molten lead to be determined for a range of temperatures

McLean and Loveday were also able to examine the merging of individual spheres once they had formed The lead spheres behaved just like the bubbles of helium in nuclear fuels which have been extensively investigated in recent years, migrating under the driving force merely of a temperature gradient The kinetics of merging of large bubbles (diameter $\sim$1 μm) was studied and found, again, to be controlled by volume diffusion of aluminium in liquid lead These large bubbles moved at a speed, independent of their size, which was consistent with the estimated temperature gradient in the microscope hot stage Bubbles with diameter less than 0 8 μm moved more slowly the smaller the bubble, this is proof positive that the motion of these small bubbles was controlled by interface energy and not by volume diffusion Very small bubbles, less than 0 12 μm in diameter, did not move at all This immobility is a new phenomenon and its origin is not clear

The observations showed the merging of pairs of large bubbles when one catches up another, and the kinetics of this process were consistent with the theory developed by earlier investigators of bubbles in nuclear fuels It seems that bubbles behave in the same way irrespective of whether they are filled with gas or with molten metal

McLean and Loveday point out that their observations have practical implications (quite apart from their relevance to the stability of composites) Free-machining alloys such as leaded brass contain small particles of soft metal in a hard alloy matrix, and it should now be possible to develop a prescription for a combined mechanical/thermal treatment to produce form of spherical dispersion with the best machining characteristics

Light from QSOs

from a Correspondent

THE QSOs—the quasi-stellar objects, or quasars—are believed by many astronomers to be remote galaxies that radiate extraordinarily large amounts of energy as a result of some violent explosion in the galactic nucleus In all probability the source of energy is gravitation, but the process whereby some of that energy is converted into visible radiation is ill-understood Although the total flux may be thousands of times greater than in a normal 'quiet' galaxy, the size of the source region must be quite small, no more than a few light days, since fluctuations in light intensity have been observed over a few days, and with a larger source they would be smeared out The investigation of possible radiation mechanisms is therefore an important task in contemporary astrophysics, and the suggestions put forward by Colvin (*Astrophys J*, **190**, 515, 1974) are of considerable interest

The popular approach, adopted by nearly all theorists, supposes that the explosive event produces a relatively dense plasma in the radiating volume (perhaps 10^8 or more ionised atoms per cm^3), a strong magnetic field linked to the plasma, and high energy relativistic electrons These are certainly the ingredients involved in producing radiation from supernova remnants like the Crab nebula, and it is attractive to try using them again with the QSOs Indeed, the most straightforward hypothesis is that the observed radiation from the QSOs, like that from the Crab nebula, is the synchrotron radiation produced by the spiralling of relativistic electrons round the magnetic field lines The relativistic electrons act as a link in the chain of energy flow energy comes to them from the explosive event and is reradiated by them as synchrotron radiation

Unfortunately, difficulties appear on a closer examination The observed spectral distribution of the radiation does not conform very well with that expected for synchrotron radiation, and in the physical conditions of a OSO one could expect the relativistic electrons to lose energy more readily through the inverse Compton process The inverse Compton process can be pictured as radiation by the electrons on account of their acceleration in the electric fields of the electromagnetic radiation through which they move, whereas synchrotron radiation is caused by the acceleration associated with spiralling round quasi-static magnetic field lines Large magnetic fields, considerably in excess of 100 gauss, are needed to ensure that the synchrotron process is dominant, and such fields are not easy to come by with some models of the 'explosive event' What Colvin has done is to suggest a two-stage process for generating the radiation from QSOs that is effective even with magnetic fields as small as 30 gauss

The basic fact made use of in the theory is that for some radiation frequencies a plasma containing a magnetic field has a refractive index greater than 1 Highly relativistic particles particles therefore move through it at a speed exceeding the speed of light in the medium In consequence they produce Čerenkov radiation—the electromagnetic equivalent of the shock wave generated in air by supersonic aircraft Colvin estimates that under QSO conditions the Čerenkov photons could have a characteristic frequency of 10^8 Hz The number of photons could be sufficient for them to make the relativistic electrons of lower energy then undergo inverse Compton energy loss and produce visible radiation of the intensity that is observed

Strength functions in rare earths

from P E Hodgson

According to the simple single-particle model of nuclei each nucleon occupies a state with definite energy and quantum numbers, and these are the eigenstates of a one-body potential Such states are selectively populated by one-nucleon transfer reactions, and a study of these reactions enables the energies of the single-particle states to be determined

Thus, for example, a (d,p) reaction simply puts a neutron into one of the unoccupied single-particle states of the residual nucleus, so that the energy spectrum of the emitted protons reflects the energies of the unoccupied states in the residual nucleus

In practice such a simple result is only found even approximately for the low-lying states in reactions depositing a nucleon into a closed shell nucleus In other cases the single-particle strength is distributed over a range of excitation energies This takes place in two different ways, either the single-particle strength can be 'spread' over a large number of states in the residual nucleus according to a Lorentzian distribution or it can be 'fragmented' by residual interactions that are not taken into account by the one-body potential The latter can result from a coupling of the single-particle states to vibrational degrees of freedom or from coupling between the one and three quasiparticle states The spreading width is expected to increase with excitation energy while the fragmentation width remains almost constant

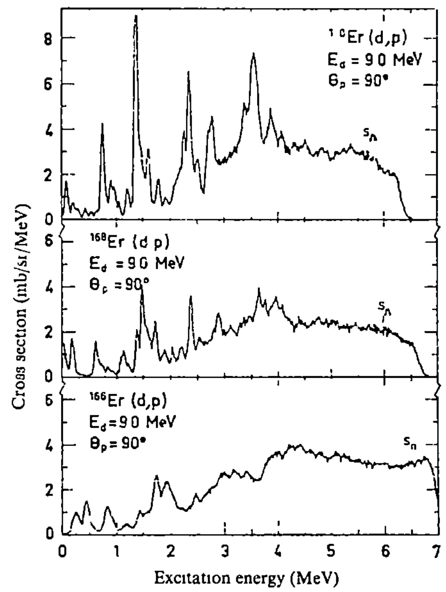

Fig 1 Energy spectrum for the (d,p) reaction at 9 MeV on three isotopes of erbium

It is important to try to find out the relative importance of spreading and fragmentation, for only if the fragmentation is less than the spreading is it possible to identify and assign quantum numbers to the higher states

This problem has recently been studied in the rare earth region by Back, Bang, Bjørnholm, Hattula, Kleinheinz and Lien (*Nucl Phys*, **A222,** 377, 1974) They irradiated several isotopes of the rare earth nuclei gadolinium, dysprosium, erbium and ytterbium with 9 MeV deuterons and measured the energy distribution of the outgoing protons Plotted on an appropriate energy scale this gives immediately a measure of the distribution of the neutron single-particle strength, or neutron strength function as it is called A typical result, that for erbium, is shown in Fig 1, the peaks in the spectra move towards lower energies for the heavier nuclei, which is what would be expected from the Nilsson model, and that there is structure below about 4 MeV but no structure at higher energies

The theoretical cross sections for these reactions were calculated using the distorted wave theory of stripping reactions to single particle states in deformed nuclei The wavefunctions were calculated as the eigenvalues of a deformed Saxon–Woods potential To enable the results of this calculation to be compared with the data the peaks were broadened by multiplying by a Lorentz factor The results for erbium are shown in Fig 2*a* and show qualitative agreement The cumulative cross section (Fig 2*b*) is in good accord with the data, indicating that the total neutron strength is preserved and that higher order processes are relatively unimportant It is, however, difficult to make unique quantum number assignments to the peaks at higher energies

This work gives a remarkably clear picture of the behaviour of neutron single-particle strengths as a function of excitation energy, and shows that both spreading and fragmentation take place

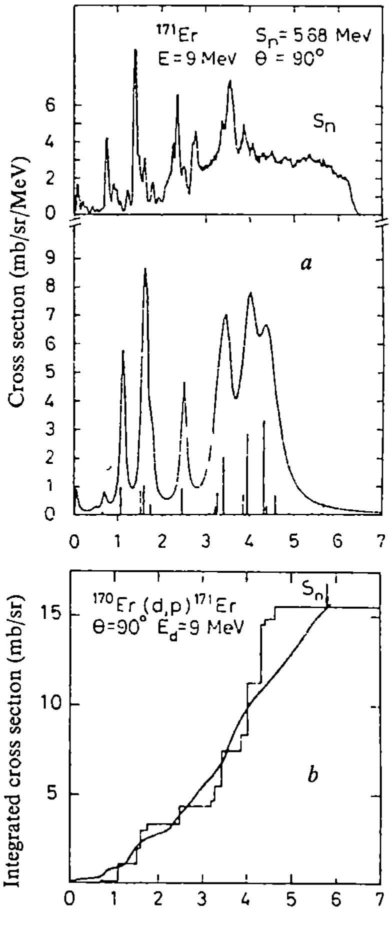

Fig 2 *a*, Experimental (upper) and theoretical (lower) differential cross sections calculated in a deformed Saxon–Woods potential The vertical lines represent the total stripping strength of the rotational band corresponding to the deformed single-particle state The smooth curve represents this strength distributed according to the Lorentz factor *b*, Comparison of the cumulative integrated experimental cross section and the theoretical cross section for energies below the neutron separation energy S_n

articles

Biological significance of the intermolecular crosslinks of collagen

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Current theories of the crosslinks stabilising the collagen fibre are based on the isolation of compounds from borohydride-reduced collagen What is the evidence for their existence in vivo *and what, if any, is the physiological significance of these proposed crosslinks both in normal and pathological conditions?*

THE recent advances in the biochemistry of collagen are due in part to its ubiquitous nature, as it exists in various forms specifically tailored to the particular function of each body tissue, and in part from the realisation that it is not completely metabolically inert but plays an important role in connective tissue disorders

The special properties that enable collagen to act as the major supporting framework of the body are largely dependent on the high structural stability of the collagen fibres Collagen fulfils this function by means of its unique molecular configuration, the highly specific alignment of the molecules during extracellular aggregation and finally by the formation of covalent crosslinks[1,2] The latter are particularly important in conferring on the fibres the high tensile strength and resistance to chemical attack necessary for their function A malfunction, genetic or otherwise, in any one of the complex sequence of events leading to the formation of the final crosslinked fibre could result in an impairment of the fibre strength There are a number of connective tissue diseases in which the clinical symptoms suggest a crosslinking defect Furthermore, the extent of crosslinking may be important in affecting the catabolic rate in the more common conditions involving fibrosis and degeneration

The initial stage in the formation of the intermolecular bonds is the oxidative deamination of specific lysine or hydroxylysine residues located in the nonhelical regions at both the N- and C-terminals of the molecules by a copper-dependent enzyme, lysyloxidase[2] The aldehydes produced are then believed to condense with reactive groups, predominantly the ε-amino groups of hydroxylysine, situated in the helical region of neighbouring molecules thus forming a network of intermolecular bonds which were assumed to be of the aldimine type[3-5] The lability of the aldimine bonds precluded the direct investigation of the compounds and the technique of mild reduction of the fibres with borohydride was therefore used to render the crosslinks stable to the hydrolytic procedures necessary for their isolation All of the major reduced components present in borohydride-treated collagen have now been characterised and from the known action of borohydride, assumptions were made as to the form of these components that are present in the native fibre

Early studies using radioactively-labelled lysine and lathyritic agents such as β-aminopropionitrile and α-amino β-thiols indicated that the reducible compounds were derived from lysine-aldehydes and were therefore implicated in the intermolecular crosslinking process[6] Despite these indications, however, the identification of compounds, or even crosslinked peptides containing these compounds, from borohydride reduced collagen does not constitute proof that their nonreduced forms act as intermolecular bonds *in vivo* since the initial condensation reaction could have been promoted by the alkaline reducing conditions

Our aim here, therefore, is to review critically the components isolated from borohydride-reduced collagen in relation to the physiological role of their nonreduced forms in the fibre The influence of these compounds on the physical properties of the fibre will be considered particularly regarding the changes that occur with age, and in pathological conditions

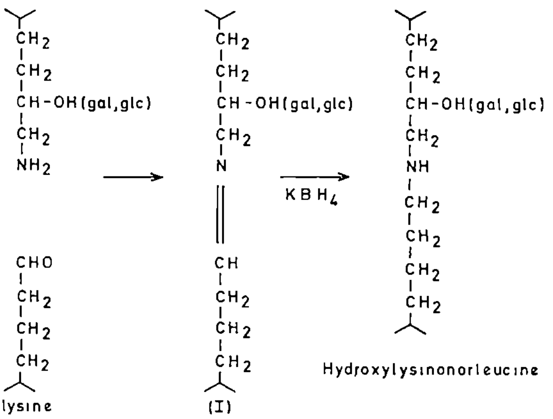

Fig 1 Biosynthesis of dehydro-hydroxylysinonorleucine (I)

Components of reduced collagen

Hydroxylysinonorleucine. This compond was the first reduced component, believed to represent a reduced intermolecular crosslink, to be isolated and characterised[7] Its presence in reduced fibres but not in solutions of monomeric collagens, and the lability of the native fibre to dilute acids, heat, and α-amino β-thiols, indicated that the nonreduced form existed as a labile intermolecular crosslink[8] Subsequent characterisation after isolation from borohydride-

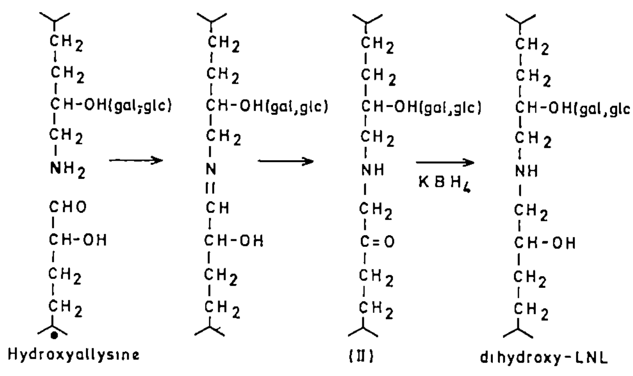

Fig. 2 Biosynthesis of hydroxylysino-5-keto-norleucine (II)

reduced native collagen was consistent with its proposed derivation from the aldimine bond form[7] (Fig 1) Unfortunately, the lability of the nonreduced form of the compound precludes the isolation of a crosslinked H-peptide from skin collagen to confirm its existence *in vivo* However, when derived from hydroxyallysine and lysine the bond is stabilised by undergoing the Amadori rearrangement (see below) It is therefore possible to demonstrate its existence *in vivo* by isolation from bone or cartilage collagen (unpublished data)

Recently, both galactosyl and glucosyl-galactosyl deriva-derivatives of the crosslink have been characterised after their isolation from alkaline hydrolysates of borohydride-reduced collagens[9] The relative proportions of the mono- and disaccharide crosslinks varies with different tissues as does the total extent of glycosylation The attachment of hexose does not seem to have a significant effect on the stability of the aldimine bond, but may facilitate aldimine bond formation by lowering the *p*K of the ε-NH$_2$ group

Hydroxylysinohydroxynorleucine. This compound designated syndesinol was reported to be a major component of borohydride-reduced tendon, bone and cartilage but was misidentified as an aldol derived from hydroxyallysine[10] Later studies by mass spectrometry indicated that it was a reduced aldimine derived from hydroxylysine[11,12] and this structure was confirmed by chemical synthesis[12] But the assumption that this compound exists *in vivo* as an aldimine was inconsistent with its unusual stability to heat, dilute acids and D-penicillamine[6,15] It was therefore proposed that migration of the double bond could take place to form the stable keto form by undergoing a spontaneous Amadori rearrangement[14] This proposal was confirmed by a Smith degradation of the reduced compound[15] isolated from bone the tritium activity was found to be associated almost exclusively with the proline and hydroxynorvaline produced, thus indicating that the rearrangement must have been complete Further studies have shown that the rearrangement is also complete in tendon and embryonic skin The crosslink must therefore exist *in vivo* as hydroxylysino-5-keto norleucine (Fig 2)

In common with the monohydroxy crosslink, glycosylated derivatives of di-hydroxylysinonorleucine are present in varying proportions with different tissues[9,16] Since the crosslink exists completely in the keto form in the native fibre, clearly the carbohydrate moeity can only be attached to the remaining hydroxyl group of the hydroxylysine residue from the helical body of the molecule

The stability of this crosslink suggested that it might be possible to isolate a crosslinked peptide from nonreduced cartilage collagen as described by Miller[17] for the isolation of the corresponding peptide from borohydride reduced fibres The peptide isolated gave an amino acid analysis consistent with a crosslinked dipeptide of α 1(II) CB4 and CB9, and on reduction revealed the presence of dihydroxylysinonoleucine[18] Periodate cleavage and subsequent reduction of the reduced H-peptide produced two peptides with analyses consistent with CB4 and CB9 Alkaline hydrolysis of the peptide indicated that about 20% of the crosslink was present as the glucosyl-galactosyl derivative

The isolation of a crosslinked peptide from nonreduced collagen provides further evidence that this hydroxy-allysine derived component does exist *in vivo* acting as an intermolecular crosslink between two adjacent collagen molecules From the order of the CNBr peptides of cartilage collagen α 1(II) chains determined by Miller[19] it is obvious that the crosslink occurs between the N-terminal telopeptide and a hydroxylysine residue in the helical region of the molecule such that the two molecules are overlapped as predicted by Hodge and Petruska[20]

It is conceivable that the acid conditions of the CNBr cleavage catalysed the Amadori rearrangements of the aldimine bond and hence, that the isolation of a peptide containing the crosslink in the keto form is not proof of its existence in the native fibre But the previous experiment in which the native fibre was reduced under alkaline conditions confirmed that the crosslink exists entirely in the keto form Thus, taken together these two experiments provide direct proof that hydroxylysino-5-keto-norleucine acts as an intermolecular crosslink in the collagen fibre under physiological conditions

Histidino-hydroxymerodesmosine. This component, present in high proportions in borohydride-reduced skin and tendon[6], has recently been shown to have a structure capable of linking three or four peptide chains[21] The component isolated is presumed to be formed by the Michael addition of histidine to the α,β-unsaturated double bond of the intramolecular aldol, and subsequent condensation of the aldehyde moiety of the aldol with hydroxylysine, the aldimine formed is stabilised by borohydride reduction thus permitting isolation of the compound (Fig 3)

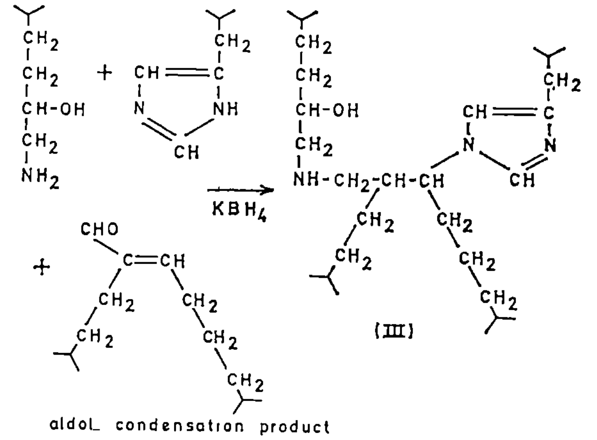

Fig 3 Formation of histidino-hydroxymerodesmosine (III)

Based on the structure of this compound, histidino-hydroxymerodesmosine, it was proposed that the compound existed before reduction as the aldimine bond form[21] Direct confirmation by the isolation of a crosslinked peptide is not possible owing to the extreme lability of the nonreduced form Indeed, it was these earlier studies on the stability of the presumed crosslink in native fibres that called into question the existence of this complex component under physiological conditions[15,22] In particular, fibres pretreated with acid phosphate not only failed to form histidino-hydroxymerodesmosine on borohydride reduction but also resulted in a concomitant increase in the amount of the reduced intramolecular aldol The proportion of the dehydro-hydroxylysinonorleucine was unaffected under these conditions These results imply that cleavage has occurred of both the aldimine bond and the Michael addition product

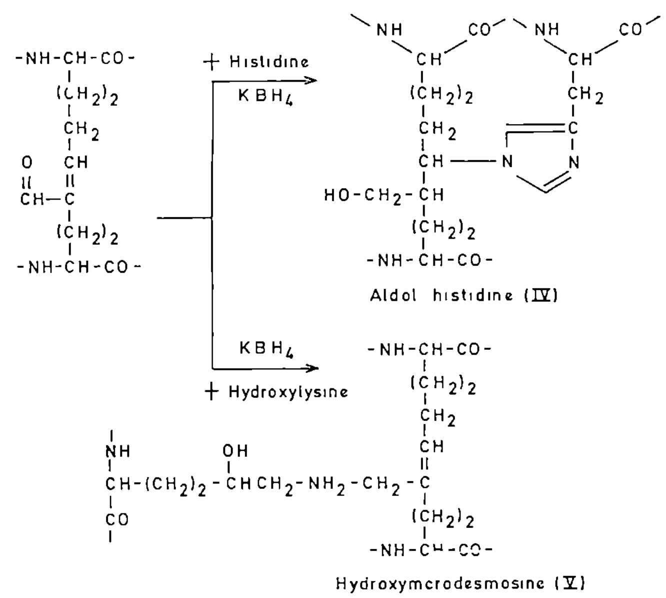

Fig 4 Formation of aldo histidine (IV) and hydroxymero desmosine (V)

But, unlike the aldimine bond linking the hydroxylysine residue, the Michael adduct forms a covalent bond which is not stabilised on reduction with borohydride but is a stable entity *per se,* as evidenced by the resistance of the histidine-aldol bond to cleavage by acid or base hydrolysis during its isolation

The most likely explanation is that the Michael addition does not occur under physiological conditions but is a base-catalysed reaction brought about during reduction with borohydride[22] Any disruption of the precise three-dimensional arrangement of the molecules in the fibre (such as freeze-drying) was found to partially inhibit the formation of this compound on borohydride reduction Thus, although it is conceivable that some noncovalent stabilisation (for example, hydrogen bonding as occurs in the active site of some enzymes[23,24]) may be derived from the adjacent functional groups in the native fibre, the results suggest that the proposed nonreduced aldimine form of histidino-hydroxymerodesmosine does not exist as an intermolecular crosslink *in vivo*

Minor reducible components

Lysinonorleucine The small amounts of this compound in reduced native collagen[25,26] probably reflect incomplete hydroxylation of the lysine residues in the helical portion of the molecule that participate in aldimine bond formation Indeed, when hydroxylation of the lysines is partially inhibited, as in vitamin C deficiency, then a higher proportion of this component is present[27] It is unlikely that the presence of such small amounts of this crosslink makes a significant contribution to the stability of the collagen fibre

Aldol-histidine and hydroxymerodesmosine These two reduced components have been isolated in small amounts and have been proposed as intermediates in the formation of histidino-hydroxymerodesmosine[28,29] (Fig 4) Since the Michael addition of histidine to the aldol is believed to be promoted by borohyde it is probable that aldol-histidine is also an artefact In view of the apparent instability of the nonreduced form of hydroxymerodesmosine and its possible conversion to the histidine derivative on reduction the rôle of this crosslink is difficult to assess, but the effect of the small proportion detectable is likely to be minimal

Hexosyl-lysine and hexosyl-hydroxylysine. The characterisation of these reducible components[30,31] which are present only as minor constituents in young tissue but which apparently increase with age demonstrated that they are derived from the condensations of lysine and hydroxylysine with glucose and mannose (Fig 5) Their structure clearly indicates that these compounds can neither be involved in collagen crosslinking nor can they bind collagen to glycoprotein since in the latter no reducing group is available for the condensation reaction Although the presence of these compounds might indicate a progressive increase with age in the binding of polysaccharides to collagen, the physiological implications, if any, of these observations are as yet unknown

Tissue differences

From the foregoing discussion it is apparent that of the components isolated from reduced collagen of young tissues, only hydroxylysinonorleucine and dihydroxylysinonorleucine are derived from intermolecular crosslinks present *in vivo* in significant amounts Clearly, the type of crosslink formed is governed by the extent of hydroxylation of the lysine residues in the N- and C-terminal nonhelical regions In skin these residues are not hydroxylated[32-34] leading to the formation of dehydro-hydroxylysinonorleucine whereas in bone and cartilage hydroxylation is virtually complete[35,36] resulting in the formation of hydroxylysino-5-keto-norleucine In tendon collagen, the lysine is about 50% hydroxylated giving rise to both these crosslinks in roughly equivalent amounts

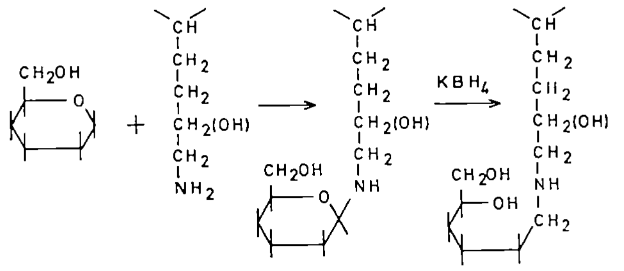

Fig 5 Formation of hexosyl-lysines and -hydroxylysines

The influence of these different crosslinks on the properties of the tissue should be reviewed in the context of their physiological function In the absence of the lysine-derived aldehydes in both experimentally induced lathyrism and copper deficiency, the collagen fibres have very little tensile strength and are completely soluble in neutral salt buffers[2] The formation of the intermolecular covalent crosslinks provides a high tensile strength to the fibre but this does not necessarily render the collagen insoluble Thus the aldimine component, dehydro-hydroxylysinonorleucine, provides a high tensile strength to rat tail tendons but the fibres are completely soluble in dilute acetic acid owing to the chemical lability of this bond For skin collagen this crosslink can account, at least in part, for the tensile strength but not for the high proportion of insoluble collagen By contrast, in the highly insoluble collagens such as bone and cartilage, the major reducible intermolecular bond *in vivo* is the stable crosslink, hydroxylysino-5-keto-norleucine, and its presence could therefore account for the insolubility of these tissues Similarly embryonic skin[37] and granulation tissue[38] possess a high proportion of this crosslink and contain very insoluble collagens But the possibility cannot be ruled out that other factors such as the presence of glycosaminoglycans may contribute to the solubility of these types of collagen

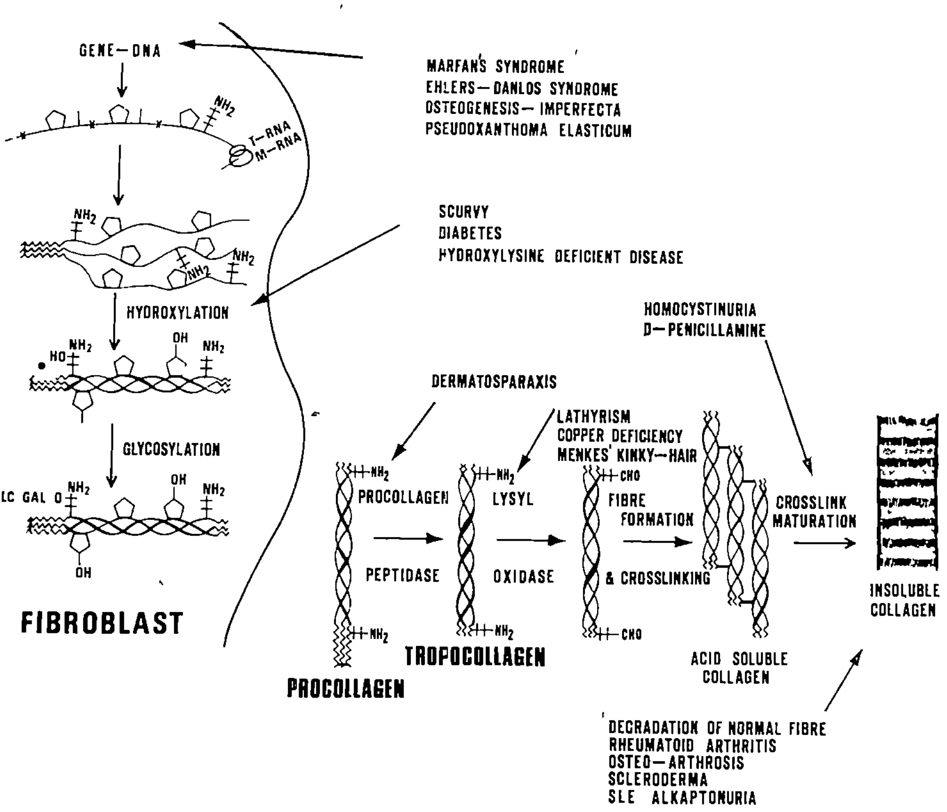

Fig 6 Schematic representation of the series of reactions in the biosynthesis of the interstitial collagen fibres depicting the possible stages at which defects could occur resulting in genetic disorders The dominantly inherited disorders, Marfans syndrome, Ehlers–Danlos Syndrome, osteogenesis imperfecta and pseudoxanthama elasticum suggest a primary structure defect[43] Scurvy results from a vitamin C deficiency leading to inefficient hydroxylation[37] Diabetes has been reported to result in excess hydroxylation[38] Hydroxylysine deficient disease is due to the absence of lysyl hydroxylase[45] Dermatosparaxis results from from the absence of procollagen peptidase[47,48], lathyrism is due to inhibition of lysyl oxidase[21] and Menkes kinky hair syndrome to copper deficiency[50], an essential cofactor of lysyl oxidase[2] Homocystine[59] and D-penicillamine act by cleavage of the labile aldimine crosslink

and any proposed relationship between the nature of the crosslink and solubility must, in the absence of further experimental evidence, remain unconfirmed As discussed below, the reducible crosslinks are known to decrease with age and it is important to emphasise that these observations only apply to young growing tissue

Age-related changes

It is well known that with increasing age collagenous tissues become increasingly more stable to external influences[39] For soft tissues such as skin, there is a progressive decrease in the amount of collagen extractable by acetic acid, a fact that is incompatible with the presence solely of the labile aldimine type of intermolecular bonds Studies of the reducible components present in tissues covering a wide range of ages showed that no new compounds other than the hexosyl-lysine derivatives are formed during the ageing process The variations with age in the proportions of the reducible crosslinks were found to parallel the rate of growth[14] Thus, there is an initial rapid rise in their amounts during the laying down of new collagen but this is followed by a gradual decrease so that at maturity the reducible crosslinks are virtually absent It is during this latter period that the collagen fibres become increasingly insoluble whilst retaining their tensile strength To account for these facts it was proposed that the reducible crosslinks act only as intermediates and are converted during the maturation process into a nonreducible and, certainly in the case of the aldimine type compound, a more stable form

One possible modification of the crosslinks that could account for the observed results is by reduction *in vivo* to form compounds identical to those produced by the borohydride treatment Such a reaction is known to occur in elastin, resulting in the formation of lysinonorleucine from its aldimine form[40] but a similar analysis of old nonreduced collagen failed to reveal any *in vivo* reduced forms[25] Later, Mechanic *et al*[41] proposed from mass spectral data that some 25 to 50% of the intermediate crosslinks in bone and dentine collagen are reduced *in vivo* and supporting evidence was derived from the *in vitro* ageing studies of Deshmukh and Nimni[42] A more recent study[14] using several different techniques has, however, confirmed the earlier report that reduction *in vivo* of the intermolecular bonds does not occur in collagen Thus the nature of the stable crosslinks present in the insoluble mature tissue remain to be elucidated, although based on the evidence now available the most likely explanation is that these stable bonds are derived in some way from the reducible crosslinks Hence, it is not necessary to propose a continuous increase in the number of crosslinks, with all the attendant difficulties of enzyme reactions within the fibre rather a spontaneous conversion of the reducible intermediates to the stable mature form would account for the observed facts

Collagen diseases

A number of heritable connective tissue disorders suggest, at least from the clinical symptoms, that the disorder might be a defect in the crosslinking mechanism (Fig 6) The Ehlers–Danlos[43, 44] syndrome in which the skin is hyperextensible, the Marfans[43] syndrome in which the aorta frequently ruptures, and osteogenesis imperfecta[43] in which the bones are extremely brittle, all strongly suggest that the disorders could be defined in terms of the molecular biology of collagen

Such definitions have recently been achieved with some recessively inherited disorders In a type of Ehlers–Danlos syndrome (Type VI) the defect was shown to be an absence of lysyl hydroxylase resulting in a collagen deficient in hydroxylysine[45] Since hydroxylysine is an essential component of all the collagen crosslinks so far identified it is not surprising that there was a consequent lack of crosslinking[46] consistent with the clinically observed tissue fragility Another enzyme deficiency occurs in Ehlers–Danlos type VII in which the low level of the procollagen peptidase activity results in the presence of procollagen during fibre formation[47] The defect may be a milder form of dermatosparaxis described in cattle in which the presence of the extra nonhelical regions disorganises fibre formation resulting in inefficient crosslinking and hence a fragile fibre[48,49] The disruption and fragmentation of the large arteries in Menkes kinky hair syndrome is probably due to the low intestinal absorption of copper[50] the absence of which is known to lead directly to inhibition of crosslinking in both collagen and elastin[51]

Although some success has been achieved with the recessively inherited disorders, the basic defect in the dominantly inherited disorders, Ehlers–Danlos, Marfans and osteogenesis imperfecta, is less forthcoming. Solubility and tissue culture studies suggest a slightly decreased level of crosslinking[52,53] but there is no dramatic change in the lysyl oxidase activity[54] or the type of crosslink in these disorders[55]

In the more common debilitating connective tissue diseases a rapid proliferation of collagen often occurs resulting in the malfunction of certain tissues as with skin in scleroderma, the joint capsule in osteoarthrosis, the synovial membrane in rheumatoid arthritis, hypertrophic scar, cirrhosis and arteriosclerosis The increased accumulation of collagen may be due either to increased synthesis, or to a decrease in catabolism, the latter being possibly due to inhibition of the collagenases or to the type of crosslink being synthesised In scleroderma the newly synthesised collagen possess the labile crosslinks of young skin[56] and in rheumatoid synovium and granulomas[38] the stable keto crosslink is formed, results which may influence rate of catabolism and subsequent treatment of the disease

In conclusion it may be stated that at least some of the reduced components in borohydride reduced collagen exist in their nonreduced form as intermolecular crosslinks determining the physical characteristic of the fibre during the rapid growth and remodelling process Further, some correlation between the type of crosslink and the pathology of the tissue is beginning to emerge The next stage should be directed towards the elucidation of the nature of the stable nonreducible intermolecular crosslinks present in mature collagen in order to further elucidate the nature of collagen in health and disease

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Infrared stars or infrareddened stars?

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Extreme interstellar reddening, accompanying visual absorption of order 100 magnitudes, is still a viable explanation for infrared objects like Becklin's star in spite of recent claims to the contrary It is unnecessary at this stage to invoke a new phase of protostellar evolution to account for them

SOURCES in regions of star formation which do not correspond to any optical stars are among the most interesting new types of object found by infrared astronomers The best known of these[1] is variously known as BN, Becklin's object and Becklin's star It has a colour temperature near 600 K Infrared sources which may be similar objects have subsequently been found in other star-formation regions, notably NGC 2264 (ref 2), Cas OB6(W3) (ref 3) and the R CrA association[4] The source in W3—called IRS 5—has a colour temperature as low as 200 K, but the other two are less extreme than Becklin's star and lie near the apexes of small conical nebulae

Becklin and Neugebauer proposed[1] that their source was a young object—perhaps a protostar—buried in a dust cocoon and that its infrared emission derived from re-radiation by the dust cloud This explanation was widely accepted until 1971 when Hyland and ourselves[5] proposed that the infrared source was the photosphere of a very luminous star reddened by the presence of extreme quantities of dust For a typical reddening law the visual absorption, A_V, must be about 70 mag

At the time there were several pieces of evidence to support that view The colours in the 1 6 to 3 5 μm region were those of a reddened hot black body rather than a cool black body and microwave observations of the Orion Nebula showed it to be sufficiently dense that A_V across it must be of order 200 mag for a normal dust to gas ratio Further, Becklin's star is immersed in an extended 20 μm source (KL, the Kleinmann–Low nebula) which is naturally accounted for as the thermal radiation of the absorbing dust This nebula is optically thick at 20 μm, also suggesting high visual extinction Because the 2 μm spectrum is featureless[5], Becklin's star must have a spectral type earlier than G and later than about B, Penston *et al* [5] favoured an F star because stars of that spectral type having the required luminosity (10^5 $L_\odot$) are known in other galaxies

In ref 5 it was predicted that the ubiquitous silicates, found in emission in a separate extended source near the Trapezium cluster[6], would produce an absorption feature in the 10 μm spectrum of Becklin's star The silicate absorption was indeed found by Gillett and Forrest[7] who also reported an absorption band at 3 2 μm due to water ice Both these features are about 1 mag deep Further support for the heavy reddening hypothesis was provided by infrared polarimetry At most infrared wavelengths, and in particular within the silicate absorption band, Becklin's star is the most strongly polarised source known A typical value for the linear polarisation is 10% (refs 8–10), scattering by dust particles is the mechanism usually invoked to explain such large polarisations

In spite of these accumulated supporting data, the heavy reddening hypothesis has been criticised recently, and even held to be no longer tenable Becklin, Neugebauer and Wynn-Williams found Becklin's star at 20 μm to be brighter by 1 5 mag than a hot reddened star fitted to the short wavelength data[11] They argued that dust accounted for the flux at 20 μm and therefore may contribute strongly at all other wavelengths They suggested that A_V is about 10 mag by comparison with the galactic centre source which they took to have an A_V of 25 mag on the basis of its 2 μm colours, and to have a silicate absorption twice as deep as that in Becklin's star

Criticism has also come from Rieke, Low and Kleinmann[12] who made high resolution maps of the region at various wavelengths in order to disentangle the contributions from BN and KL By combining their derived fluxes with data at longer wavelengths, Rieke *et al* deduced the luminosity of the KL nebula to be about 5×10^4 $L_\odot$ If the Kleinmann–Low nebula absorbs most of the energy of the underlying star it must essentially have the luminosity of that star The figure 5×10^4 $L_\odot$ is too low to be reconciled with the best available photometry of Becklin's star

This situation is worsened by the discovery[12] that Becklin's star is only one of several similar sources that cluster within the KL nebula Some of these sources were found by Wynn-Williams and Becklin[13] and given different designations In Table 1 we list all the infrared sources known in the Orion Nebula There are two distinct types most are compact objects less than 2 arc s diameter, but three are extended and can be attributed to thermal radiation from hot gas (free–free emission) or cool dust It is not clear from present data whether two of the 'compact' sources, IRc4 and IRc5, really are compact or are small condensations in the Kleinmann–Low nebula According to Rieke *et al*, IRc2, 3, 4 and 5 are redder than Becklin's star and probably have deeper silicate absorptions

It is important to clarify the essential issue underlying this controversy about the value of the absorption (which we will continue to measure by A_V while noting that it is in fact determined at 2 μm) If A_V lies in the range 65 to 70 mag, the unreddened source can be explained by a normal star in the temperature range 5,000 to 12,000 K Such stars are consistent with the 2 μm spectrum On the other hand if A_V is less than 45 mag the unreddened source has a colour temperature below 1,500 K and the radiation must originate principally from dust grains No consistent model exists with A_V between 45 and 65 mag In the second case Becklin's star may be an example of a new class of prestellar object On the evidence available so far, must such new objects be invoked or can an explanation in terms of normal stars be retained?

It seems most unlikely that A_V can be as low as the 10 mag suggested by Becklin *et al* [11], since there is no sign of anything like such strong silicate or ice absorption in stars, such as Cyg OB2 12 (ref 14) and MWC349 (ref 15), with $A_V \sim 10$ The comparison with the galactic centre is of dubious weight since the 2 μm galactic centre source (on which A_V is based) and

Table 1 Infrared sources in the Orion Nebula

Type of source	Designation		Probable A_V (mag)	Notes
	ref 12	ref 13		
Definitely compact (stars)	IRc1	BN	70	
	IRc2		100	included in IRS 3?
	IRc3	IRS 3	160	
		IRS 2	40	
		IRS 4	?	near NA
Compact or dust concentrations?	IRc4	core of KL	≤200	
	IRc5		?	seen only at 21 μm
	IRe2	KL	50–200	
Extended	IRe1		small	free-free component
		NA	small	Trapezium

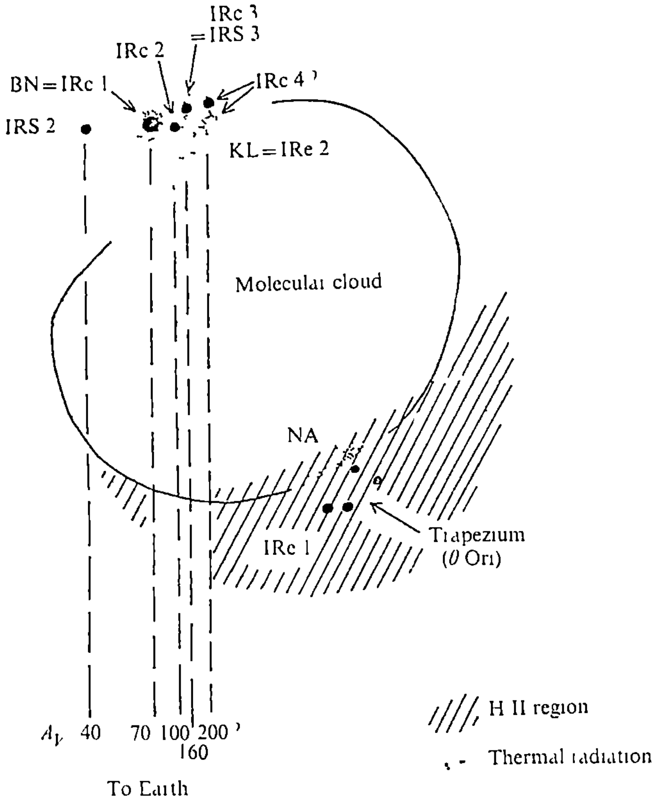

Fig. 1 Schematic diagram of M42 sliced through position angle 145°–325° The solid boundary to the neutral cloud is intended to represent not the limit of the material but a contour above which the density is very high IRc5 and IRS 4 are not plotted because of the uncertainty of their nature

the 10 µm source (with silicate absorption) have different structure[16] In general an extended source will tend to show smaller absorption at shorter wavelengths if the absorbing medium is clumpy Further, although the ratio of the equivalent widths of the silicate bands in the galactic centre and Becklin's star is about two, a more sophisticated analysis[17] including the effect of emission shows the column density of silicates to be more nearly the same

The choice between an A_V of 30 to 45, consistent with Rieke *et al*'s model of a cluster of protostars completely enveloped in the Kleinmann–Low nebula, and an A_V of about 70 is not so easily made We wish to point out that the latter is by no means untenable and that only slight modifications to the original version of ref 5 are necessitated by the large number of new data We must now explain (i) the 20 µm excess and (ii) the discrepancy in luminosity between the KL nebula and the cluster of stars

The 20 µm excess

There is no difficulty in finding a mechanism to produce the 20 µm excess Re-radiation by cool (~ 200 K) dust, or possibly free–free emission by circumstellar gas, can provide the necessary 20 µm flux without significantly affecting the near infrared colours An explanation based on dust at such a temperature would not require the source to be more extended than 1–2 arc s Dust re-radiation would also tally with the silicate emission implied in ref 17

On the other hand the dereddened 5–20 µm colour and the gradient of the 8–13 µm continuum fit a free–free slope Such a free–free component could not, however, originate in an ionised hydrogen shell without the simultaneous emission of a Brackett γ line, sufficiently intense to have been detected by Penston *et al*[5] Cool ionic free–free, as considered by Dyck and Milkey[18], is an outside possibility

The luminosity

Because insufficient luminosity is re-radiated by the KL nebula, it cannot completely surround the stars We therefore now envisage a cluster lying at the far side of the Orion Nebula just as the Trapezium cluster lies on the near edge (Fig 1) There would not necessarily be any manifestations at optical wavelengths of the presence of such luminous stars outside the nebula and essentially unobscured from some aspects In particular any Lyman continuum radiation is soaked up by the circumstellar gas so these stars would be incapable of ionising more distant nebulosity

It is relevant that very luminous F and G type stars are seen associated with star-formation regions in the Large Magellanic Cloud[19] Luminosities run up to $10^6\,L_\odot$ as in HD268757 which is associated with the nebula N83 Thus a star of $10^5\,L_\odot$ is by no means exceptional We are not privileged to examine the star-formation regions in our own galaxy face on, these extremely bright stars might well be hidden behind dust clouds

A more severe problem arises in the case of IRS 5 in W3 if this source too is to be explained as a highly reddened star To account for the depth of the silicate feature[17] we need only assume the ratio of silicate to total absorption to differ marginally from that of Becklin's star But the near infrared photometry implies a total luminosity of $\sim 3\times10^6\,L_\odot$ even if we choose what seems to be the more reliable distance of 2 kpc for Cas OB6 (ref 20) rather than the value of 3 kpc used to date in discussions of this infrared star Like Becklin's object, the reddened star must have a 20 µm excess A multiple system of very luminous stars could account for this object also, an alternative is a truly remarkable (and possibly unstable) single object of the type discussed by Hoyle, Solomon and Woolf[21] That W3 IRS 5 must also lie at the back of its absorbing cloud is implied by the 100 µm flux reported for this source[22] which at 2 kpc corresponds to a luminosity of $5\times10^5\,L_\odot$ Most or all of the flux can be accounted for by other sources in W3 (such as IRS 2 of ref 3), so IRS 5 may lie so far back of the absorbing cloud that there is essentially no re-radiation

The Kleinmann–Low nebula

Returning to discuss the details of the Orion Nebula sources, we note that in our model the Kleinmann–Low nebula is also seen through dust and could have an A_V as high as 200 mag Gillett and Forrest[6] suggested that the optical thickness of the silicate absorption in the KL nebula is much greater than in Becklin's star, implying a higher A_V The polarisation is also higher[10] Unfortunately, the interpretation of these data is complicated The two source picture (BN+KL) was extant when these

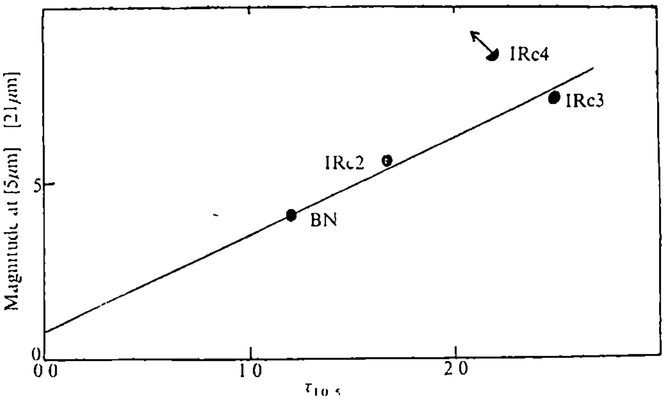

Fig 2 A plot of the 5 µm to 21 µm colour index of the compact infrared sources in M42 against the depth of the silicate feature below a reddened continuum fitted at 5 and 21 µm The data are from ref 12 This correlation can be explained if the sources have similar intrinsic 5–21 µm colour indices and are reddened in proportion to the silicate absorption

observations were made, but now we know of many sources Establishing how much each source contributes to each observation is impossible without a very accurate knowledge of the size, profile, separation and position of the beams at the time of observation The uncertainty has only minor effect on the results for Becklin's star itself, but could greatly distort the results for the extended source

Observational tests

It is not easy to distinguish between the 10,000 K, 70 mag model and the ~1,200 K, ~ 30 mag model One method would be to measure interferometrically the diameter of Becklin's star at 5 μm where the long wavelength component is negligible and scattering should not much increase the apparent size A baseline of 500 m would be needed

An alternative would be to deduce the relationship between A_V and the depth of the silicate or ice absorption For example more stars like Cyg OB2 12 and MWC349, and possibly the galactic centre, should be observed in this way The form of the interstellar absorption curve in the near infrared should follow from these observations as well as the depths of selective absorption features If infrared sources like those in NGC2264 and R CrA do fit into this scheme they may be most valuable as intermediate cases since the optical nebulae probably caused by reflection[4,23] indicate the presence of hidden stars The ratios of continuous to selective and of optical to infrared absorption are unlikely to be constant throughout the Galaxy, so this process is best done for the immediate vicinity of the Orion Nebula, where there may be no suitable sources From the data given in ref 12 we may, however, deduce a relationship between the reddening at 5 μm and the apparent depth of the silicate feature In Fig 2 we have plotted the 5 μm to 21 μm colour index in magnitudes against the optical depth at 10 5 μm in the silicate band The tight correlation exhibited by BN, IRc2 and IRc3 is further evidence for the presence of reddening The limit on IRc4 does not fit this correlation, but we have already noted (Table 1) that this source may be of a different nature It is interesting that IRc3 seems to possess double the absorption found in BN The intersection of the best fit line with the [5 μm]–[21 μm] axis gives the intrinsic colour of the sources, assuming them all to be similar, this is seen to be small For a typical theoretical reddening law $A_V \sim 50 E_{[5]-[21]}$, but this seems to be an overestimate since it suggests $A_V > 100$ mag for BN, and this figure could be further raised if there is any silicate absorption at 21 μm Improved data might lead to a more reliable diagram of this kind Essentially identical conclusions follow from interpreting Fig 1 of ref 24 in the same way

A final test would be to obtain a better 2 μm spectrum of Becklin's star to search for normal stellar features, for example Brackett γ With the improvement in signal/noise ratio likely to be brought about by the introduction of InSb detectors, this is an attractive proposition

Even if Rieke *et al*'s model for the Orion sources is correct, absorptions with $A_V \geqslant 30$ mag shroud any optical counterparts from our view Such dense clouds and high absorptions were unanticipated before the advent of infrared astronomy they are now a recognised part of the process of star formation

We thank Dave Aitken and Gareth Wynn-Williams for discussions

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Catalytic head assembling protein in virus morphogenesis

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About 250 molecules of a 'scaffolding protein', not found in mature phage, catalyse the assembly of about 420 coat protein subunits into a precursor shell containing both proteins in concert with DNA encapsulation, all the scaffolding molecules exit from the precursor shell to take part in further rounds of the same process

SINCE Fraenkel-Conrat and Williams demonstrated that tobacco mosaic virus could be reconstituted from its purified protein and nucleic acid components[1], it has been widely believed that virus parts specify their own correct assembly Caspar and Klug[2] provided a geometrical model for the self assembly of spherical virus capsids as the aggregation of similar subunits into quasi-equivalent positions on the surface of an icosahedral shell There is

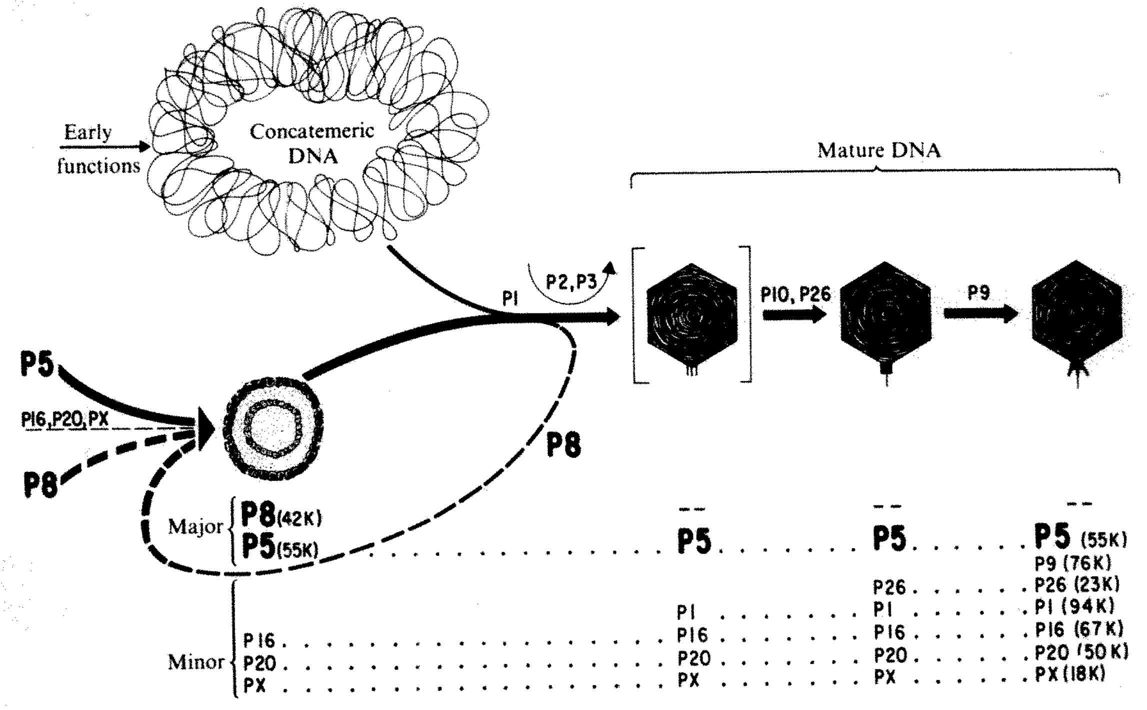

Fig. 1 Pathway of phage P22 head assembly and DNA encapsulation. This diagram summarises results from Botstein *et al*[17], King *et al.*[18] and this article. A prohead shell lacking DNA is the first identifiable structure in the pathway. It contains two major protein species, the products of gene *5* and gene *8*. (The products of genes *16* and *20*, though ordinarily incorporated into proheads, are not needed for morphogenesis.) With the incorporation of the product of gene *1*, and the function of the gene *2* and *3* products, the prohead encapsulates and cuts a headful of DNA from the concatemer DNA molecule, and concurrently P8 exits from the particle without proteolytic cleavage. The newly filled head is unstable (it loses the DNA) until acted upon by the gene *10* and *26* products, after which the complete head is converted to a phage particle by addition of the tail parts (gene *9* protein) as shown by Israel *et al.*[37]. P before a gene number refers to the polypeptide of that gene. The polypeptide chains found in each structure are listed below it, with their molecular weight shown in the last column. Brackets around the first filled head structure designate that it is unstable and loses DNA on isolation. The nature of the replicating DNA in P22-infected cells has been described by Botstein and Levine [22]; Tye *et al.*[16] have shown conclusively that P22 chromosome maturation proceeds by a "sequential headful cutting" mechanism[31].

now strong evidence that at least simple viruses conform to this pattern, and can self assemble from the separated components of the mature virus[3–5]. Recently, however, experiments with the more complex bacteriophages λ and T4 have shown that some proteins are required for assembly which are absent from the mature virus[6–9]. In addition proteolytic cleavage[5,8,10–12], and even covalent fusion[13] of structural proteins have been found to accompany the assembly of various viruses and bacteriophages. We describe here a protein that specifies the correct dimensions of a virus, catalytically, by temporarily complexing with the major coat protein to form a transient precursor shell of the virus. The dissociation of the protein from the precursor shell is associated with DNA condensation.

P22 is a temperate phage which infects the bacterium *Salmonella typhimurium*[14]. Its head is isometric, 600 Å in diameter, with a small spiked tail plate attached (Figs 1 and 2*b*), and contains double-stranded DNA of molecular weight 27×10^6 (ref. 15). The sequence present at one end of the chromosome is repeated at the other end to the extent of about 2%, and the genome is circularly permuted with respect to the chromosome so that the ends are not unique[15,16]. The DNA metabolism of infected cells, the genetic control of virus assembly, and the protein composition of virus particles have been described in detail by Botstein and coworkers[16–22]. For our purposes the important feature of the life cycle (Fig. 1) is the formation of a 240S head precursor structure, the prohead (Fig. 2*c* and *d*), which contains the major coat protein—the product of gene *5*—and a second major protein not found in mature phage, the product of gene *8*. (Proheads also contain three minor protein species, the products of genes *1*, *16* and *20*, but these are not required for prohead formation[17,18].)

The prohead is the precursor head structure in the cutting and packaging of a mature length chromosome from the overlength replicating DNA. This process requires the products of genes *1*, *2* and *3*; if any of these proteins are missing (due to mutation), proheads and uncut DNA accumulate[17]. The immediate product of DNA encapsulation is unstable until acted on by the products of genes *10* and *26*. If these proteins are missing the DNA comes back out, leaving empty heads, distinguishable from proheads by morphology and sedimentation. If cells are infected with *amber* mutants defective in gene *8*, most of the coat protein remains unassembled, or assembles into aberrant aggregates[18]. Since P8 is needed for the assembly of the coat protein into phage, but is not found in phage, we termed it a 'scaffolding protein'[18]. The P22 prohead is similar in this respect to tau particles of phage T4 described by Kellenberger *et al.*[23] and Simon[24]; Showe and Black[9] and Laemmli and Favre[25] have shown that such particles contain proteins needed for morphogenesis but absent from the mature phage.

We show here that the isometric capsid of phage P22 does not self assemble. Roughly 200–300 molecules of the scaffolding protein catalyse the accurate polymerisation of the coat protein into a precursor shell containing both proteins. Upon DNA encapsulation, all the scaffolding protein molecules depart from the precursor shell. These then proceed to catalyse the polymerisation of newly synthesised coat protein molecules into another precursor shell.

Protein composition of proheads

Proheads are the earliest shell structures detectable in the head assembly pathway[18]. We isolated proheads in high concentration from infected cells, separated their proteins

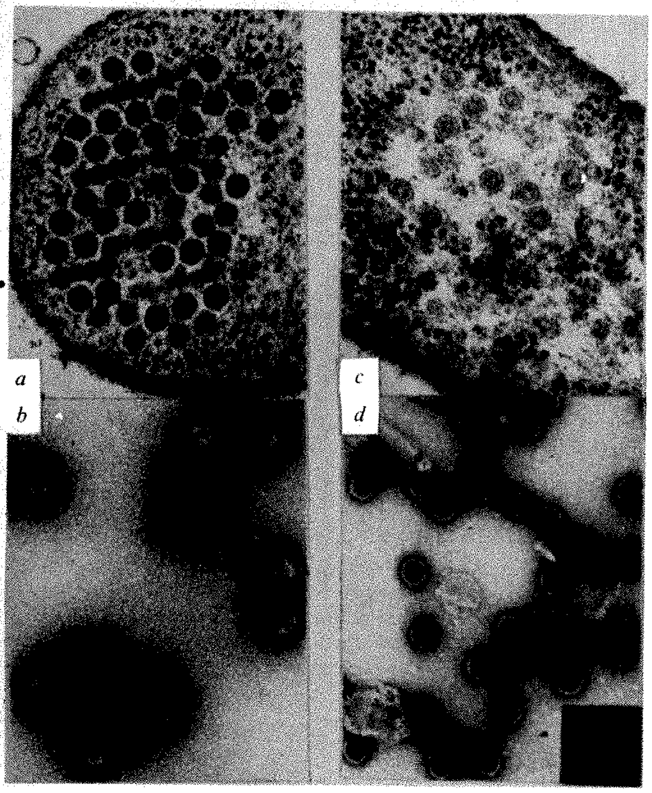

Fig. 2 Morphology of phage head structures. The upper panels are electron micrographs of ultrathin sections of P22 infected Su$^-$ DB21 (ref. 19) cells, prepared by the method of Simon[24]. Each lower panel is an electron micrograph of particles (negatively stained) isolated from cells infected with the same phage as the panel above it. *a* and *b*, Normal phage particles; *c* and *d*, proheads from cells infected with phage carrying an amber mutation in gene *2*. These structures contain both the coat protein and the scaffolding protein. The phage also carried the clear mutation c_1[7] to prevent lysogeny and a lysis defective mutation in gene *13* (*am*H101)[17]. A more detailed account of the *in vivo* morphology of the various head-related structures formed during P22 infection will be presented later (Lenk, Casjens, Weeks and King, in preparation). The magnification of all panels is ×60,000, and the magnification of the insert in panel (*d*) is ×140,000.

by electrophoresis through SDS acrylamide gels, and determined mass ratios by staining with Coomassie brilliant blue and fast green. We first used cells infected with *amber* mutants defective in genes *1*, *2* or *3*, since proheads are the only particulate phage structures (240S) formed in them. Proheads from mutant-infected cells are qualitatively indistinguishable from the precursor proheads isolated from wild type-infected cells[17,18]. Proheads were isolated from crude lysates by centrifugation through a sucrose-caesium chloride step gradient, and then further purified by centrifugation through a sucrose gradient, as described in the legend to Fig. 3. An electron micrograph of the resulting preparation is shown in Fig. 2*d*. Figure 3*h* (bottom right) shows the proteins of a *2*$^-$ prohead (proheads from cells infected with an *amber* mutant in gene *2*) separated on an SDS gel and stained with Coomassie brilliant blue, with a densitometer tracing above. P8 is a major component. Proheads isolated from *1*$^-$, *2*$^-$, and *3*$^-$-infected cells were very similar in protein composition (except that the *1*$^-$ proheads lacked P1, as expected[18]).

The results of many gel analyses are summarised in Table 1. Taking 420 as the number of coat protein subunits[17], and assuming equivalent binding of the stain to the two species of protein, we calculated that the proheads accumulating in *1*$^-$, *2*$^-$, and *3*$^-$-infected cells contain about 260 molecules of P8. In the lysates from which these particles were isolated, more than 90% of P5 and P8 molecules were in proheads.

To determine the ratio of scaffolding protein to coat protein in proheads from productive infected cells, we infected a culture with P22 and isolated proheads from samples lysed 22, 51, 90, and 109 min later. Figure 3*a–d* shows that P8 is a major component of these precursor structures. However, wild-type infected cells also contain empty heads, which lack P8 (ref. 18). These sediment at 170S and contaminate the 240S prohead fractions. From electron micrographs of negatively stained samples of the isolated wild type proheads, we estimated the contamination to be about 30%. Table 2 gives the number of P8 molecules in proheads isolated from productively infected cells, corrected for contamination by empty heads. The

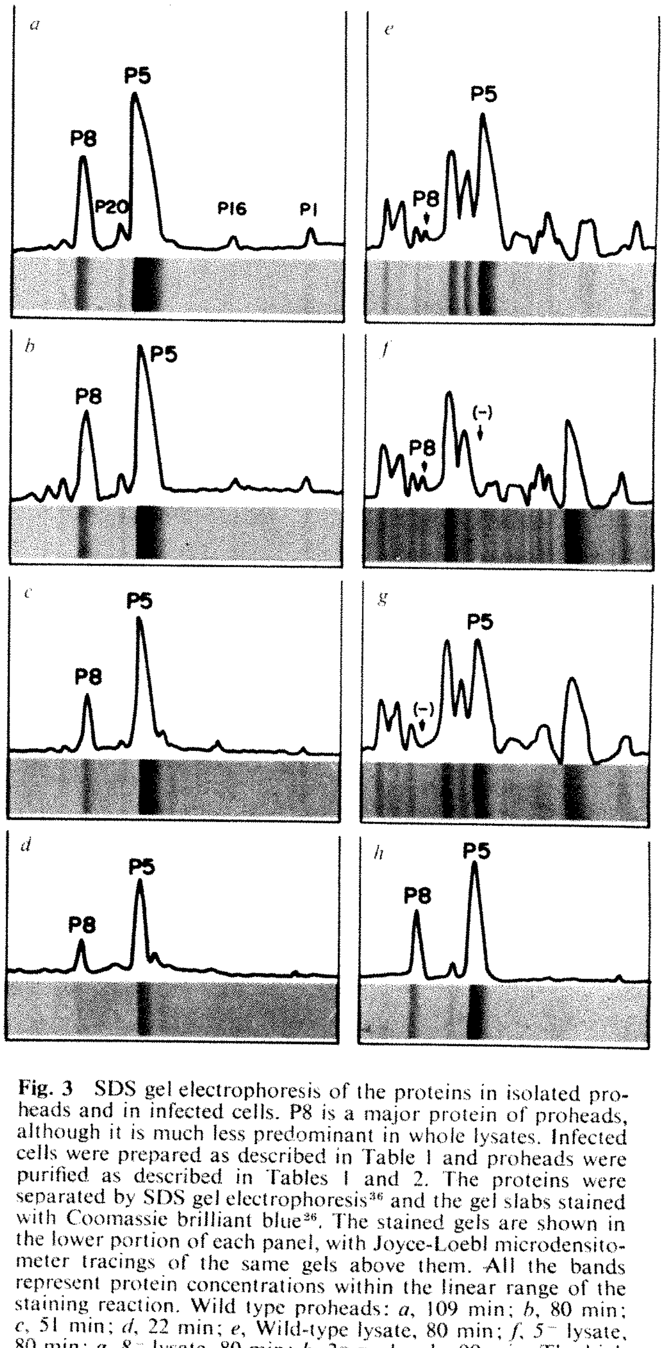

Fig. 3 SDS gel electrophoresis of the proteins in isolated proheads and in infected cells. P8 is a major protein of proheads, although it is much less predominant in whole lysates. Infected cells were prepared as described in Table 1 and proheads were purified as described in Tables 1 and 2. The proteins were separated by SDS gel electrophoresis[36] and the gel slabs stained with Coomassie brilliant blue[26]. The stained gels are shown in the lower portion of each panel, with Joyce-Loebl microdensitometer tracings of the same gels above them. All the bands represent protein concentrations within the linear range of the staining reaction. Wild type proheads: *a*, 109 min; *b*, 80 min; *c*, 51 min; *d*, 22 min; *e*, Wild-type lysate, 80 min; *f*, *5*$^-$ lysate, 80 min; *g*, *8*$^-$ lysate, 80 min; *h*, *2*$^-$ proheads, 90 min. The high molecular weight regions of the gel are to the right. The band which is present in increased amounts in the *5*$^-$ and *8*$^-$ lysates is P9, the tail protein[17,37]. Gene *9* is subject to a control system different from the other late proteins (Lew and Botstein, unpublished experiments): it is overproduced in cells infected with mutants blocked in DNA encapsulation. This phenomenon is independent of the overproduction of P8 described later in the text.

Table 1 Protein composition of proheads

	Stain	Molecules of P8 per 420 P5 molecules*
1^- Proheads	Coomassie blue	259±16
	Fast green	245± 8
2^- Proheads	Coomassie blue	266±16
	Fast green	not done
3^- Proheads	Coomassie blue	270± 7
	Fast green	251±10
++ Proheads†	Coomassie blue	239±25
	Fast green	252±20

Proheads were prepared as follows cultures of the su$^-$ bacteria DB 21 (ref 19) were grown to 2×10^8 cells per ml in broth[20] at 37° C, infected with P22 carrying the clear marker c_1[7], a lysis-defective mutation in gene *13* (*am*H101) and an *amber* mutation in gene *1* (*am*N10), gene *2* (*am*H200), or gene *3* (*am*N6)[17], at a multiplicity of five After 90 min of aeration, cells were pelleted, resuspended in about 0 002 volumes of 0 02 M Tris-HCl, 10^{-3} M $MgCl_2$, *p*H 7 5, *containing chloroform (to induce lysis)* and pancreatic DNase (to reduce viscosity) The cell debris was removed by low speed centrifugation, and the clear, opalescent supernatant was layered on a 15–30% sucrose gradient and centrifuged at 20,000 r p m for 2 h in an SW 27 1 rotor Opalescent prohead bands were removed by piercing the side of the tube with a syringe The proteins of the purified proheads were separated by SDS polyacrylamide slab gel electrophoresis[35 36] The resulting gels were stained with Coomassie brilliant blue[26] or fast green[27], and tracings of the coloured bands were made with a Joyce-Loebl microdensitometer The ratio of copies of P8 to P5 was calculated from the areas under the peaks after correcting for the molecular weight differences All measurements were made *within the range in which stain density was proportional* to the amount of sample applied to the gel

* Average of a minimum of four polyacrylamide gel experiments The errors are the calculated standard deviations

† Corrected for the fraction (15–30% among 250–750 particles counted on electron micrographs) of contaminating empty heads (which contain no P8) The mutant prohead preparations contain no empty heads

ratio of scaffolding protein to coat protein in these precursor *proheads did not change significantly throughout* infection, and was similar to the P8/P5 ratio in proheads isolated from *1*$^-$, *2*$^-$, *and 3*$^-$-infected cells The slightly higher P8/P5 ratio of the wild-type proheads may be due to overestimation of the contamination by empty heads

Since the amino acid composition of these proteins is unknown, we do not know if P8 and P5 bind the same amount of dye per mg of protein Published results indicate small differences in binding for different proteins[26 27] We therefore conclude that the prohead intermediates in phage P22 assembly contain 200–300 P8 scaffolding protein molecules for every 420 P5 coat protein molecules

The ratio of P8 to P5 in proheads is quite different from the ratio of the two proteins in the total culture, as can be seen by comparing the lysate gel patterns of Fig 3*e* with the prohead patterns P22-infected cells accumulate very little P8 with respect to P5 The *5*$^-$ and *8*$^-$ mutant lysates (Fig 3*f*,*g*) provide positive identification of the two proteins

Figure 4 shows the rates of synthesis for the two proteins during infection, determined from the extent of incorporation of ^{14}C-labelled amino acids during short pulses At late times 15–20 copies of P5 coat protein were synthesised for every copy of P8 synthesised

Botstein *et al*[17] showed that there was no detectable cleavage of late proteins in P22-infected cells We have *performed further* pulse-chase experiments to look for proteolytic cleavage of P8, using shorter pulse times—75 s at 30° C Samples withdrawn from the culture at various times after chase were lysed immediately with chloroform, mixed with sodium dodecyl sulphate (SDS) sample buffer, and frozen before electrophoresis through an SDS gel slab Recovery of radioactive P8 (weight of peak from densitometer tracing of autoradiogram) after the chase was as follows *15* s, *56* mg, 2 min, 67 mg, 5 min, 73 mg, 9 min, 82 mg, 13 min, 73 mg, 18 min, 79 mg We conclude that P8 is not cleaved to lower molecular weight during the phage life cycle Since P8 is not in phage, and *is not degraded, it must be removed from proheads intact*

P8 recycled during morphogenesis

If 200–300 molecules of P8 are required to make a prohead, and therefore a phage, and if each molecule of P8 were to take part in the formation of only one prohead, then Figs 3 and 4 indicate that too few molecules are made to account for the yield of viable phage particles Pulse-chase experiments[18] suggested that P8 is reused during infection, that is, after release from a prohead taking part in DNA encapsulation, the P8 molecules take part in further rounds of prohead assembly If this is true, then P5 coat protein molecules should chase from proheads into phage, but the P8 scaffolding protein should remain with the prohead fraction, complexed with newly synthesised coat protein molecules

To test this prediction, infected cells were exposed to radioactive amino acids continuously from 12 min after *infection (which is before the onset of late protein syn*thesis) to 28 min after infection, so that all the molecules of P8 and P5 synthesised up to that time would be radioactive At 28 min excess unlabelled amino acids were added to stop the uptake of labelled amino acids At various times measured from the start of the chase (1, 2, 4, 7, 11, 16 and 22 min) samples of the culture were shaken with chloroform to lyse infected cells, and aliquots of the lysates were centrifuged through sucrose gradients, first to separate phage particles (500S), and then at higher speed to separate proheads and empty heads (240S and 170S) The radioactivity in the prohead and empty head peaks decreased with time after the chase, and the radioactivity in the phage peak increased, as expected[18] (data not shown) To follow the fate of P5 and P8 molecules individually, we dissociated the prohead, empty head, and phage-containing sucrose gradient fractions in hot SDS, and electrophoresed the samples through an SDS gel slab The resulting autoradiograms displayed the expected band patterns[18] proheads contained P5 and P8 as the dominant bands, plus much smaller amounts of radioactive P1, P16 and P20 In comparison the phage lacked the P8 band but displayed tail protein (P9) and P26 and PX The empty heads were similar to the proheads except they also lacked P8 Figure 5 shows that labelled coat protein chased from proheads and empty heads into phage, while labelled scaffolding protein remained associated with the prohead fraction Since the ratio of P5 and P8 in proheads does not change during infection (Table 2) the radioactive P8 must have associated with nonradioactive coat protein

Table 2 Number of molecules of P8 in head structures isolated at various times after infection

	Time after infection			
	22 min	51 min	80 min	109 min
Proheads	295±30	290±25	315±30	300±25
Empty heads	< 10	< 10	< 10	< 10
Phage	< 10	< 5	< 2	< 2

Errors represent the limits of at least two determinations Particles were purified and values for P8 determined as described in the legend of Table 1

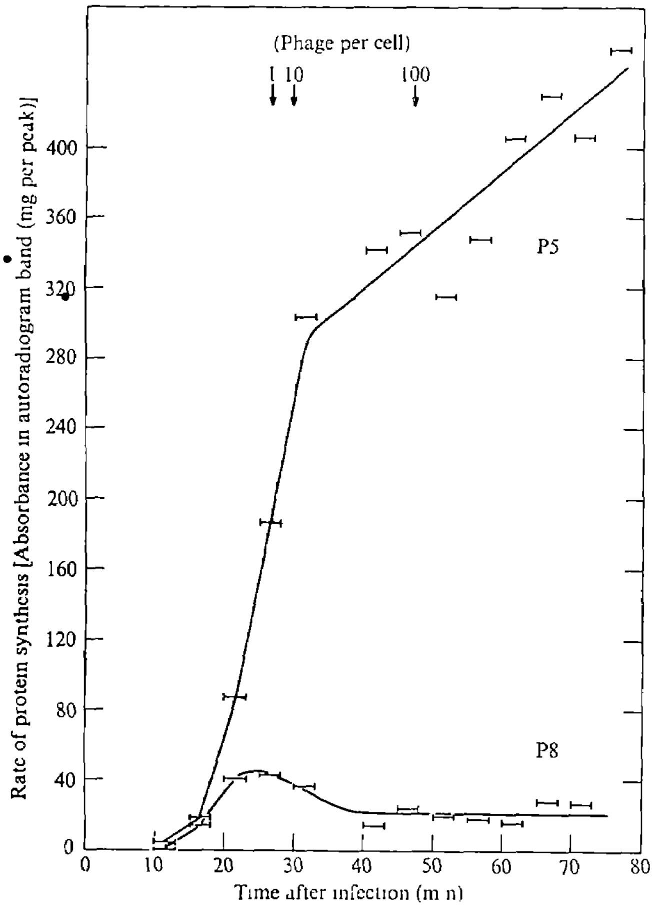

Fig 4 Rate of P8 and P5 synthesis in phage-infected cells *S typhimurium* D21 was grown in M9 medium[17] at 37° C to 2×10^8 cells per ml and infected with five $c_1 13^-$ phage per cell At the times shown, samples of the culture were incubated with ^{14}C-labelled amino acid mixture to give a final concentration of 0 2 μCi ml^{-1} Three minutes later incorporation was stopped by addition of excess casamino acids, and after a further 1 5 min incubation, the cells were chilled in ice and then centrifuged at 4,000 r p m for 10 min The resulting pellet was resuspended in 1/4 the original volume and lysed by shaking vigorously with chloroform Samples were heated in SDS sample buffer and the proteins separated by electrophoresis through a 12% SDS polyacrylamide slab gel[35 36] Densitometer tracings of appropriately exposed autoradiograms were prepared[38] and the peaks representing the bands of radioactive P8 and P5 cut out and weighed Experiments performed at 30° C gave similar results, as did experiments in which cells were lysed immediately after the chase of the radioactive amino acids

synthesised after the chase The simplest interpretation of these results is that on encapsulation of DNA by the prohead, all the P8 molecules dissociate from the complex, and rapidly reassociate with more coat protein to form further proheads (These proheads would also be expected to contain newly synthesised unlabelled P8, which would not have been detected in the experiment)

Suppose a prohead is formed of both previously synthesised and newly synthesised scaffolding protein, but only newly synthesised coat protein, then after short pulses of label, the ratio of labelled P8 to labelled P5 in proheads should decrease for pulses given later and later during infection, since the pool of unlabelled recycling P8 will increase with respect to the pulse labelled P8 To test this prediction infected cells were exposed to 3-min pulses of radioactive amino acids at various times after infection Two and one half minutes after the chase of radioactivity, samples were lysed and centrifuged through sucrose gradients to separate proheads This was sufficient time for much of the pulse labelled P5 and P8 to form proheads, but not long enough for substantial label to appear in phage[18] The prohead proteins were separated by SDS gel electrophoresis and autoradiograms prepared and tracings made from them The molar ratio of P8 to P5 radioactivity in pulse labelled proheads was as follows 33 min, 21%, 44 min, 9 9%, 51 min, 7 8%, 60 min, 5 2% The ratio of labelled P8 to P5 in proheads clearly decreased as the infection progressed Since the total protein composition of these particles was not different (Table 2), we ascribe the decrease to the presence of unlabelled recycling P8 synthesised before the pulse period

How many rounds of prohead assembly does each P8 molecule participate in, on the average? To answer this question we measured the total amount of P5 and P8 in the whole lysate by Coomassie brilliant blue staining of SDS gels, and also measured the P5 and P8 in the proheads, empty heads, and phage fractions isolated from the lysate Given that it takes about 250 molecules of P8 for the incorporation of 420 molecules of P5 into phage, we calculated how many times the P8 in the culture at the time of lysis would have had to function, to account for the amount of coat protein assembled into particles (phage + empty heads + proheads) present in the culture at the time As Fig 6 shows, this number increases during infection and is about 5 at the time the cells would have lysed (45 min) if the phage were not carrying a lysis delay mutation

Since this is a somewhat unusual catalytic protein, in which the formation of the product requires the concerted reaction of more than two hundred catalyst molecules, we have not expressed the data in Fig 6 as an enzymatic turnover number The value corresponding to that is about 0 2 phage per minute per 250 P8 molecules at 37° C

Prohead assembly

The experiments described here show that about 250 molecules of the 42,000 molecular weight gene *8* scaffolding protein catalyse the assembly of about 420 molecules of the 55,000 molecular weight gene *5* coat protein into a precursor prohead shell containing both proteins The scaffolding protein then exits from the prohead in concert with the condensation of the phage DNA, yielding a packaged chromosome and free scaffolding protein, which then repeats the cycle Proheads could be described as giant enzyme–substrate complexes, no scaffolding protein is detectable in virus particles containing DNA, the product of the reaction

That proper shell formation depends on the participation of the scaffolding protein is shown most clearly by the analysis of gene *8* mutants cells infected with amber mutants defective in gene *8* synthesise normal amounts of coat protein (Fig 3*f*) but this protein does not assemble efficiently into closed shells[17 18] Instead, aberrant aggregates are formed, among these are shells with smaller diameter than phage, and larger 'spiral' structures This latter class looks like a shell which has failed to join its growing edges, and then kept adding subunits at the exposed edge Nested shells have also been seen We have performed pulse-chase experiments with 8^--infected cells which show that P5 assembles into aberrant structure less than 1/5 as efficiently as the P5 in wild-type infected cells assembles into proheads These experiments suggest that the scaffolding protein increases both the rate and accuracy of shell formation

What is the structure of the prohead complex? The electron microscopic appearance of proheads in both negatively stained specimens and ultrathin sections of infected cells shows organised internal structure (Fig 2*c*, *d*) The images suggest either a double shell structure, or a ball within a shell Since the outer shell has dimensions similar to the mature phage, and also represents more mass than the inner material, we presume that the outer shell is made of coat protein, and the inner material represents the scaffolding protein The two proteins could be intimately associated as a single thick shell, and the appearance in thin sections could be due to an artefact of fixation or staining Since the capsid of the mature phage is most likely a lattice with icosahedral symmetry[2], it seems probable that the prohead also represents an icosahedral type surface lattice Much less clear is the symmetry expected of the scaffolding protein organisation

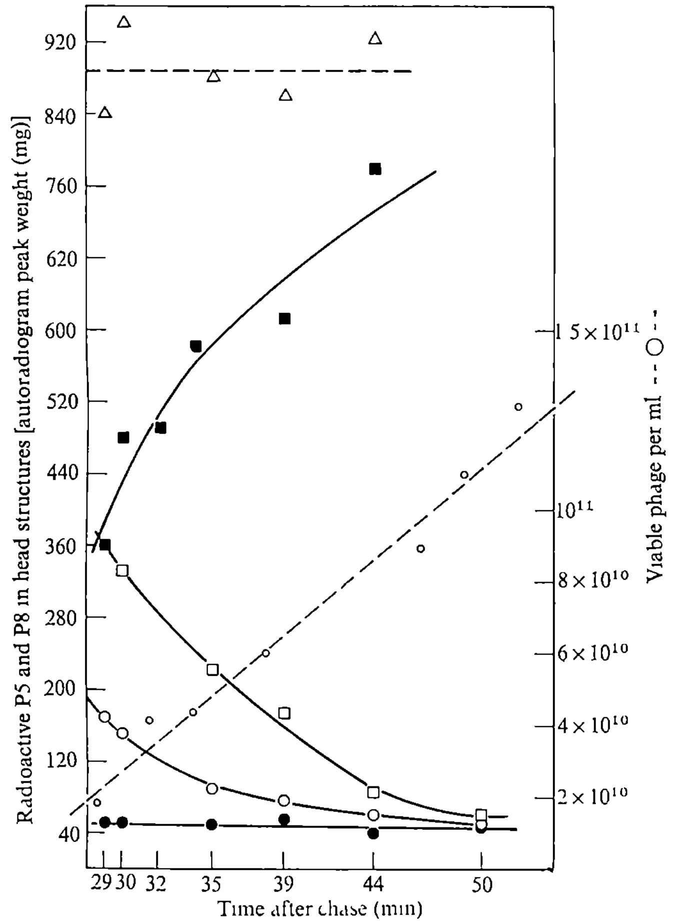

Fig 5 Continued association of labelled scaffolding protein with proheads during phage growth This figure summarises the fate of labelled coat and scaffolding protein after a chase of radioactivity which had been present continuously from 12 to 28 min after infection The amount of labelled P5 in the phage fraction (■) increases after the chase while the amount of labelled P5 in the prohead (○) and empty head fractions (□) decreases with time after the chase However, all of the labelled P8 associated with proheads (●) at the time of the chase remains associated with proheads even though these structures are serving as intermediates in assembly and the P5 associated with them is converted to phage That viable phage are continuously forming is shown by the dashed line The open triangles show the sum of the labelled P5 from the various fractions Though not shown in the figure, the minor proteins present in proheads P1, P16 and P20 also chased from the prohead fraction into the phage fraction during the chase The experiment was performed as follows D21 was grown in M9 medium[17] at 30° C to 2×10^8 cells per ml and infected with 10 c_1*13*$^-$ phage per cell At 12, 16, 20 and 24 min after infection, 2 5 µCi of ^{14}C-labelled amino acid mixture was added per ml of culture (giving a final concentration of 10 µCi ml^{-1}) At 28 min excess casamino acids were added and at 1, 2, 4, 7, 11, 16 and 22 min thereafter, samples were lysed by shaking vigorously with chloroform Incorporation of radioactivity into trichloroacetic acid insoluble material was linear during the labelling period For separation of phage, 0 25 ml aliquots were centrifuged through 5–20% sucrose gradients in 0 01 M Tris buffer, with a shelf of 0 3 ml of CsCl, ρ - 1 6 g cm^{-3} Centrifugation was for 20 min at 20,000 r p m , 20° C For separation of proheads and empty heads (*a* and *c*) 0 1 ml aliquots were centrifuged for 30 min at 30,000 r p m , 20° C Radioactivity was determined by mixing aliquots of the gradient fractions with Triton X-100/toluene based scintillation fluid and counting in a scintillation counter Particles from the sucrose gradient separations were dissociated in SDS sample buffer and electrophoresed through a 10% SDS polyacrylamide slab gel and the radioactive bands determined by autoradiography Densitometer tracings of the autoradiograms were made and the peaks cut out and weighed

How does the scaffolding protein control the assembly of the coat protein? The scaffolding protein itself might assemble into a large complex which serves as a template or 'assembly core' for coat protein polymerisation, as suggested[9,23] for phage T4 If that were the case we would expect to find such complexes in cells infected with coat protein amber mutants We find, however, that all the scaffolding protein in *5*$^-$ lysates sediments as free subunits (<4S) We have also been unable to see organised structures in thin sections of *5*$^-$-infected cells

These observations suggest that the scaffolding protein complexes not with itself initially, but with coat protein, and that a coat protein/scaffolding protein multimer is the functional unit in shell formation Presumably in the absence of the scaffolding protein, the bond angles between coat protein molecules are not specified rigidly enough to ensure that over a 1,900 Å circumference the edges will join properly Multimers of coat protein and scaffolding protein would be expected to have much larger reactive surfaces and thus be able to specify bond angles more accurately

Figure 4 shows that P8 is synthesised at a low level in wild-type infected cells We have also measured the synthesis of late proteins in cells infected with amber mutants in each of the P22 late genes The pattern of P8 and P5 synthesis is the same as wild type in cells infected with mutants which are capable of DNA packaging (*16*$^-$, *20*$^-$, *10*$^-$, *26*$^-$ and *9*$^-$) But, cells infected with mutants defective in genes, *1*, *2*, or *3* overproduce P8 4–8-fold In these cells P8 is trapped in the prohead complexes which accumulate and is not recycled The overproduction is not due simply to the large pool of unpackaged DNA that accumulates, since *5*$^-$-infected cells do not overproduce P8 Various experiments on the rate of P8 synthesis in mutant infected cells (unpublished experiments) indicate that the level of P8 synthesis is a function of the concentration of unassembled P8 Its synthesis is thus self-regulated, similar to the *Salmonella* histidine repressor[28], threonine deaminase[29] and T4 DNA polymerase[30] In the case of phage head assembly, this provides a feedback loop between DNA packaging and protein synthesis, if encapsulation is proceeding efficiently, P8 is released to form further proheads, and depresses its own further synthesis

Since P8 is a major component of proheads, its catalytic function saves the cell considerable protein synthetic capacity, the economy is even greater when the synthesis is regulated so that the protein is produced at a rate just sufficient for optimal assembly

P8 and DNA condensation

Although we have focused on its scaffolding function, the DNA condensation function of P8 might be of equal biological importance Since all phage head structures containing DNA lack P8, the 200–250 molecules of P8 must exit from the prohead before or during DNA encapsulation This unusual exchange reaction requires the products of genes *1*, *2*, and *3*, since if any one of them is missing, proheads and unpackaged DNA accumulate[17] P2 and P3 are not associated with proheads or phage, we suspect that they interact with precursor DNA, perhaps forming a binding site for proheads The actual condensation of the DNA within the head might be directly coupled with the exit of P8 According to Laemmli's model for DNA packaging[25], for example, the exit of P8 might expose charged sites within the head which serve to collapse the DNA The scaffolding protein might exit by

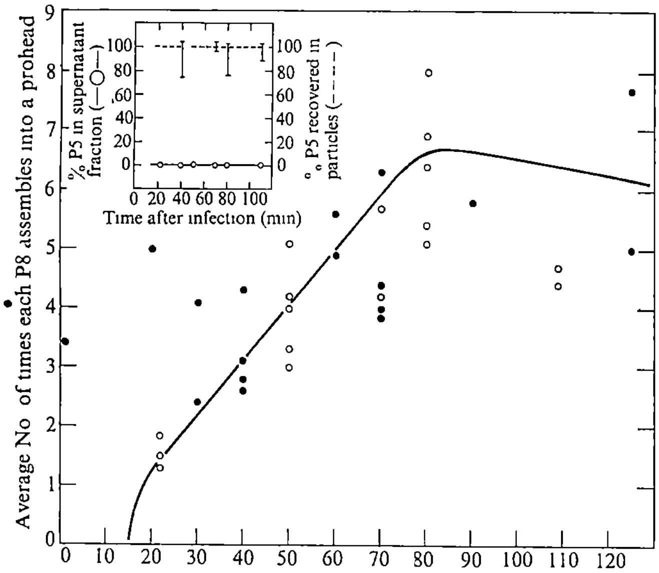

Fig 6 Recycling of scaffolding protein molecules The graph shows the average number of rounds of prohead assembly that each scaffolding molecule has taken part in during phage growth The calculation was as follows

$$\text{number of times P8 reused} = \frac{(\text{P8/P5})_{\text{prohead}}}{(\text{P8/P5 F})_{\text{cells}}}$$

where F is the fraction of the total P5 molecules in the cell which is in phage or phage precursor structures F was found to be very near 1 0 (see below) The open and closed circles represent two independent experiments *S typhimurium* DB21 was grown in broth to 2×10^8 cells per ml and infected with P22 $c_1 13^-$ at a multiplicity of 10 At the times indicated samples of the infected cells were pelleted, resuspended in 0 1 volume of electrophoresis buffer, heated to 100° C for 2 min, and the proteins were separated by SDS slab gel electrophoresis (see Fig 3) The protein bands were quantitated by tracing with a Joyce-Loebl microdensitometer, and the areas under the P8 and P5 peaks measured At various times after the infection described above, samples were also taken for analysis of the phage-specific structures present The infected cells were concentrated by centrifugation, lysed with CHCl$_3$, layered on the top of a sucrose gradient with a high density (1 6 g cm^{-3}) CsCl cushion, and centrifuged for 45 min at 45,000 r p m in a Beckman SW 50 1 rotor The fraction containing all the particles (phage, proheads and empty heads), that is, the top of the CsCl cushion, and the supernantant (top 1 5 ml) fraction were removed with a syringe, and the proteins separated and quantitated as above The insert in the figure above shows the results of such an analysis normalised to the total amount of P5 present

rearrangement of the coat protein shell over its entire surface, or through a single channel If the exit of P8 molecules is directly coupled to the entry of DNA, then the scaffolding protein catalyses not only shell formation, but also DNA condensation, since each molecule takes part in as many cycles of DNA packaging as it does of prohead formation

Since the P22 chromosome is packaged by a 'headful cutting' mechanism[16,17,31], the length of the chromosome must be determined by the size of the head Thus the scaffolding protein, in specifying accurately the dimensions of the prohead shell, is specifying the length of the virus chromosome

Proheads are similar in morphology to the T4 tau particles described by Kellenberger *et al*[23], Simon[24] and Laemmli[32] showed that these are precursors to phage, and Showe and Black[9] demonstrated that the inner material is probably an assembly core composed of the product of T4 gene *22* product and internal proteins The *22* product and internal protein III are cleaved down to fragments during DNA packaging[25] Thus, the P22 scaffolding protein and T4 assembly core are formally equivalent, in the former case the assembly core is removed by proteolytic cleavage during DNA packaging and in the latter case the protein is removed intact and then recycles Bacteriophages T7 and T3 may be assembled similarly to P22 since during infection a top component occurs which contains a protein absent from phage[33,34] This protein is not cleaved during assembly, and is specified by a gene which maps adjacent to the coat protein, just as in P22

Catalysis of morphogenesis

The first case in which a protein was shown to catalyse a step in morphogenesis was the attachment of T4 tail fibres to particles by gene *63* product (Wood and Heninger[6]) The gene *63* product is typical of an enzyme in that a stable complex with the two substrates could not be isolated We think the scaffolding protein represents a new kind of catalytic function (*a*) hundreds of protein molecules are necessary for the formation of one product of the reaction, (*b*) the catalyst molecules become a stable component of the immediate product of the reaction—in essence the scaffolding protein specifies the correct dimensions of the coat protein shell by temporarily becoming part of the shell, (*c*) the dissociation of the complex is a very complicated reaction requiring at least three other proteins, plus the encapsulation of a viral chromosome

Perhaps most important, these results show clearly that proteins critical for assembly may not be found as part of the mature structure[6–8,25] For example, the proteins which condense DNA into chromosomes in higher organisms may not be found in the mature chromosome We would also like to emphasise that although a major component of a viral precursor, the P22 scaffolding protein — because of its ability to recycle — is only a minor component of phage-infected cells We suspect that many structure formation processes will turn out to proceed by a similar combination of catalytic with self-assembly mechanisms

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Operator and promoter are separate in the tryptophan operon in *Salmonella typhimurium*

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Studies of the trp *mRNA made by* Salmonella typhimurium *strains carrying* trpOA *and* trpOAB *multisite mutations have shown that these are deletions They do not extend into the* trp *promoter* (trpP1) *as shown by their ability to recombine with* trpP4 *mutation Thus the* trp *operator* (trpO) *and the* trp *promoter are seperable and arranged in the order* P1-O-A

In a preliminary report[1] we presented evidence that in the *trp* operon of *Salmonella typhimurium* the promoter (*trpP1*) and operator (*trpO*) were separable and arranged in the order *P1-O* in relation to the first structural gene *trpA* (Fig 1) Part of this evidence was the observation that we were unable to find *supX-trpO* deletions but easily obtained multisite mutations with the characteristics of deletions extending from *trpA* to *trpO* and leaving *trpP1* intact We did not, however, exclude the possibilities that these multisite mutations were (*a*) insertions of extraneous genetic material[2,3] containing a functional promoter, or (*b*) deletions extending beyond *trpP1* and joining the remainder of the *trp* operon to an outside promoter We report here that our multisite mutations are indeed *trpA-trpO* deletions which do not extend fully into *trpP1*, thus strengthening our earlier conclusions about the order and separability of *trpP1* and *trpO*

The isolation of our multisite mutations was based on the observation that strains carrying strong polar mutations in *trpA* or in *trpB*-region I (Fig 1) grow very slowly on anthranilic acid supplement since they have very low levels of PRPP-phosphoribosyl transferase (PRT (EC 2 4 2 18) activity[4] Secondary mutants of such strains selected for improved utilisation of anthranilic acid, a substrate of PRT, cary deletions of various portions of the *trpA* gene and, depending on the mutants, *trpB*-region I (refs 4,5) Representatives of this class are *trpAB688* and *trpAB674* in Fig 1 By selecting simultaneously for normal growth on anthranilic acid supplement and resistance to 5-methyltrptophan (5MT), an analogue of tryptophan able to act as a corepressor[6], we obtained exclusively multisite mutations extending from *trpA* to *trpO* (ref 1) These are represented by *trpOA526, trpOAB673* and *trpOAB1349* in Fig 1 Since these presumptive deletions correct for a polar effect by increasing the level of activity of a growth limiting enzyme (PRT), they should end 'in phase' By transducing the mutant *cysB403* and the double mutant *cysB403* Δ*trpPOABEDC167* with lysates grown on each multisite mutant and plating on tryptophan supplemented minimal medium, we determined that all multisite mutations cotransduced with *cysB* at the same frequency as the wild type *trp* operon and were not translocations of the distal segment of the operon to the vicinity of some other promoter on the *S typhimurium* chromosome

We have been able to look at the *trp* transcripts of our *S typhimurium* mutants by taking advantage of the observation of Denney and Yanofsky[7] that *S typhimurium*

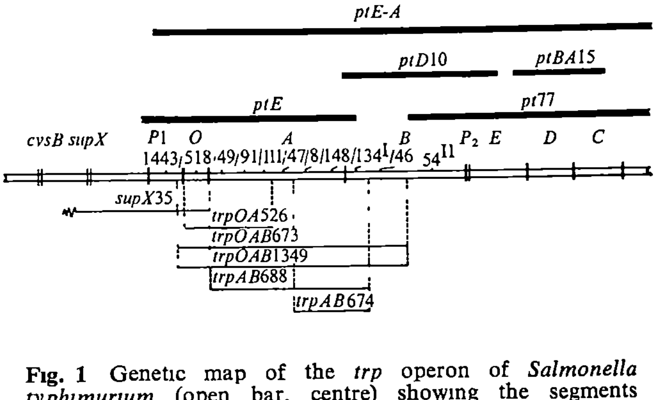

Fig. 1 Genetic map of the *trp* operon of *Salmonella typhimurium* (open bar, centre) showing the segments covered by the various multisite mutations used in this investigation (below bar) and the corresponding portions of the *trp* operon of *Escherichia coli* carried by transducing Ø80 pt phages used in hybridisation experiments (closed bars above map) The *trp* operons of both *E coli* and *S typhimurium* consist of five closely linked structural genes under tryptophan control[10,11] The order of these genes is the same in both organisms but the nomenclature is different that is the first structural gene, which codes for the anthranilate synthetase (AS) component of the AS-PRT complex, is designated *trpE* in *E coli* and *trpA* in *S typhimurium*[10,11] The numbers above the bar represent point mutations of the early portion of the operon and are shown in their map order[1,5,8] P1 represents the main *trp* promoter and P2 is the low-efficiency internal promoter-like element[21,27] The map indicates both regions of *trpB*, the gene for the anthranilate-PRPP-phosphoribosyl transferase (PRT) component of the AS-PRT enzyme complex region I codes for the glutamine transferase activity of this component, and region II for the PRT activity proper[26] The isolation of the multisite mutations is described in the text The isolation of *supX35* was described by Margolin and Bauerle[27]

Table 1 Levels of *trp* mRNA and tryptophan synthetase (TS) in mutant strains

		Repressed		Derepressed					
		^{3}H–RNA (% of input c p m) hybridised to DNA of	TSase relative specific activity	^{3}H–RNA (% of input c p m) hybridised to DNA of					TSase relative specific activity
Strain	Genotype	*pt E–A*		*ptE*	*pt77*	*ptBA15*	*ptD10*	*pt E–A*	
—	*trpD9*	0 008	0 5	0 416	0 735	0 33	0 39	1 215	119
SO–160	*trp0A526*	1 022	90	0 012	0 554	0 39	0 41	1 048	85
SO–441	*trp0AB1349*	0 249	6	0 011	0 264	0 20	0 21	0 429	3 2
SO–584	*trp0AB673*	0 367	6	0 016	0 441	0 23	0 25	0 470	3 2
SO–631	*trpAB674*	0 010	1 2	0 080	0 592	0 47	0 49	1 160	118
SO–645	*trpAB688*	0 005	1 25	0 010	0 560	0 46	0 49	1 040	123

The nomenclature used for the bacterial strains follows the recommendations of Demerec *et al*[15] For pulse labelling experiments cells (8 × 10^8 ml^{-1}) grown at 37° C in L broth were collected, washed twice with cold Vogel and Bonner's minimal medium[16] supplemented with 1% glucose and 0 25% casamino acids and resuspended in the same medium at an approximate concentration of 2 × 10^9 cells per ml Tryptophan (50 μg ml^{-1}) was added (repression conditions) or omitted (derepression conditions) as needed A 10 ml sample of the cell suspension was incubated at 30° C on a shaker for 10 min and then pulse labelled for 2 min with 10 μCi ml^{-1} carrier-free ^{3}H-5, 6-uridine (37 Ci mmol^{-1}) Labelling was stopped and the cells killed by pouring the culture immediately on to an equal volume of crushed frozen medium consisting of 0 01 M Tris-HCl buffer (*p*H 7 3), 5 mM $MgCl_2$, 10m M NaN_3, 400 μg ml^{-1} chloramphenicol, and 20% sucrose RNA was prepared by the phenol extraction method of Okamoto *et al*[17] except that the treatment with 0 5% sodium dodecylsulphate (SDS) and phenol was carried out at 4° C The RNA was precipitated with ethanol, dissolved in 0 5 M KCl, 10m M Tris-HCl buffer (*p*H 7 3) and filtered through a Millipore filter (0 45 μm pore size) After being further precipitated and redissolved by the same procedure, this purified ^{3}H–RNA was hybridised with denatured DNA of phage φ80 and the transducing phages described in Fig 1 30–50 μg of ^{3}H-RNA were used for each assay Bacteriophage DNA was prepared essentially as described by Morse and Yanofsky[18], with some modifications, heat denatured at 100° C for 20 min, rapidly cooled and bound to Millipore filters of 0 45 μm pore size (10 μg per filter) as described by Gillespie and Speigelman[19] and modified by Morse and Yanofsky[18] Hybridisation of ^{3}H-RNA to the filter-bound DNA was carried out in 0 4 ml volumes of 1× SSC (0 15 M NaCl, 0 015 M trisodium citrate) The reaction mixtures were incubated for 18 h at 65° C and cooled slowly to below 40° C The filters were then washed twice with cold 2× SSC, incubated for 30 min at 60° C in this buffer and rinsed twice with cold 2× SSC to reduce nonspecific binding, dried and the radioactivity determined in a liquid scintillation counter using a toluene base scintillation mixture No RNase treatment was used since this decreased considerably the number of counts hybridised in heterologous mRNA–DNA mixtures[7] The results given have been corrected by subtracting the counts retained on control φ80 DNA filters (about 20 c p m) Total radioactivity of ^{3}H-uridine incorporated into RNA (total ^{3}H-RNA) was measured as counts incorporated into cold TCA-precipitable material (~ 10^6 c p m) Tryptophan synthetase (TS) was assayed according to the procedure of Smith and Yanofsky[20] The β component of this enzyme was assayed in all strains except *trpD9*, known to lack it For this strain the α component was assayed Since both components of the tryptophan synthetase complex are repressed and derepressed in a coordinate fashion[21,22], the level of either of them can be used as an index of the level of expression of the *trp* operon Cells for the enzyme assays were grown in minimal medium[16] containing 0 5% glucose and 2 μg ml^{-1} casamino acids and supplemented with different amounts of L-tryptophan 50 μg ml^{-1} for repressing growth conditions, and 5 μg ml^{-1} for derepressing growth conditions Protein was determined by the method of Lowry *et al*[23] One unit of specific activity is defined as the utilisation of 0 1 μmol of substrate (indole) per mg protein at 37° C in 20 min[20] Specific activities are relative to those of repressed wild type (specific activity = 0 4) taken as unity

trp mRNA can hybridise to *E coli trp* DNA carried by transducing φ80 *pt* phages if certain modifications are introduced into the standard hybridisation procedure Under our conditions, hybridisation in the heterologous *S typhimurium* mRNA–*E coli* DNA system was about 67–75% of that in the homologous *E coli*–*E coli* system in good agreement with the results of Denney and Yanofsky Table 1 summarises some properties of our multisite mutants and the control strain *trpD9*, which carries a nonpolar mutation in the *trpD* gene All the strains in which the multisite mutation extends into the operator make tryptophan synthetase (TSase, EC 4 2 1 20) and *trp* mRNA constitutively, while in those which have an intact operator this enzyme and *trp* mRNA are under tryptophan control Table 1 also shows that the multisite mutants, (with the exception of *trpAB674*), have no detectable mRNA hybridising to the DNA of the phage *ptE*, but they do have detectable levels of mRNA hybridising to *pt77*, *ptBA15* and *ptD10* as well as *ptEA* DNA Since *ptE* carries only the DNA of the first structural gene of the operon, these are the results we would predict if the multisite mutations were deletions of the segment indicated in the genetic map (Fig 1) We would anticipate that *trp0A526* and *trpAB674* might contain some *trp* mRNA hybridisable to *ptE* DNA, but found a very small amount of it only in *trpAB674* We do not understand this observation yet, but it could be related to the decreased homology between mRNA and DNA in our heterologous system In any case, there is a distinct difference between *trpD9* and our multisite mutants in their content of *trp* mRNA hybridisable to *ptE* DNA

The *trp* mRNA of *E coli* is about 6,700 nucleotides long[8] and has a sedimentation coefficient of 33S in 5–30% linear sucrose gradients[9] Since the *trp* operons of *S typhimurium* and *E coli* are identical in overall organisation and the sizes of their protein products[10,11], we expect that the *trp* mRNA of *S typhimurium* will have the same sedimentation coefficient as that of *E coli* when centrifuged under the

Table 2 Transcription times (min) of various segments of the *trp* operon after the addition of rifampicin

		Transcription times (min) of segments hybridised to DNA of				*trp* mRNA			
Strain	Genotype	*ptE*	*ptD*10	*ptBA*15	*ptEA*	Estimated no of nucleotides	Molecular weight	Predicted S values	Observed S value
—	*trpD9*	1 0	2 25	4 7	4 7	6700	2×10^6	33	33
SO-160	*trp0A526*	0	1 16	4.0	4 0	5800	1 7×10^6	29	30
SO-441	*trp0AB1349*	0	1 0	3 4	3 4	4800	1 4×10^6	26	25
SO-584	*trp0AB673*	0	1 0	3 5	3 5	5100	1 5×10^6	27	23
SO-631	*trpAB674*	0 7	1 8	4 0	4 0	5800	1 7×10^6	29	27
SO-645	*trpAB6888*	0	1 5	3 4	3 4	4800	1 4×10^6	26	24

The experiment was carried out as described in the legend to Fig 3 Estimated sizes for the *trp* mRNA are based on transcription time. A value of zero indicates that no detectable ^{3}H-mRNA was found to hybridise to a particular *pt* DNA The predicted S values were obtained from a standard curve of S against molecular weight of various species of RNA based on figures obtained from the literature The observed S values were determined by the procedure described in the legend to Fig 2 same conditions This is indeed the case, as shown in Fig 2, for the *trp* mRNA of *trpD9* The mRNA molecule at the peak of the resedimentation profile (Fig 2*b*) sedimented at about 33S This *trp* mRNA could hybridise to *ptE* as well as *pt77* DNA, as expected from a normal polycistronic *trp* mRNA Figure 2*c* shows the resedimentation profile SO-584 (genotype *trpOAB673*) In contrast to *trpD9* the largest *trp* mRNA of this mutant sediments at 23S and does not hybridise to *ptE*, indicating that it is a transcript of only the distal portion of the operon The *trp* mRNAs of all our multisite mutants had smaller sedimentation coefficients than that of the control (Table 2), as expected if they contain deletions in the *trp* operon

Measurements of transcription time further support the idea that our multisite mutations are deletions of the proximal portion of the *trp* operon Rifampicin inhibits the initiation of transcription but does not interfere with transcription which is in progress A plot of the rate of ^{3}H-uridine incorporation after addition of rifampicin is linear[12] in agreement with the assumptions that (*a*) the distribution of RNA polymerase molecules transcribing the operon under derepression conditions is random, and (*b*) all polymerase molecules move at a constant rate Transcription time can then be obtained by extrapolation of the line expressing the rate of ^{3}H-uridine incorporation to the time axis[12] As Fig 3 shows, it takes about 4 66 min to transcribe the *trp* operon into *trpD9* For a mRNA which is 6,700 nucleotides long this corresponds to a transcription rate of 24 nucleotides per s, quite close to that calculated for *E coli* (25–28 nucleotides per s)[12] The figure also shows that the transcription time for the small deletions *trpOA526* and *trpAB674* is shorter (4 min) than that for *trpD9*, and that for the larger deletions *trpOAB637*, *trpOAB1349* and *trpAB688* is shorter still (about 3 5 min) Estimates of the size of the *trp* mRNA of each mutant based on these data are given in Table 2, and predicted S values for the respective *trp* mRNAs are compared with the observed ones The agreement between the estimated and the observed values is reasonably good

If ^{3}H-RNA is hybridised to the DNA of *pt* phages carrying different segments of the *trp* operon, such as *ptE*, *ptD10* and *ptBA15* (Fig 1) we can obtain additional evidence that the deletions are limited to the beginning (*trp-OAB*) portion of the operon Thus, if the first structural gene or parts thereof are missing, transcription of the second structural gene should end earlier in the mutant strains than in the control Results from these experiments are shown in Table 2 In *trpD9* synthesis of mRNA for the first gene (portion hybridisable to *ptE* DNA) is completed in 1 min, that for the second gene and early portion of the third (portion hybridisable to *ptD10 DNA*) is completed in 2 25 min, and the mRNA for most distal portion of the operon (hybridisable to *ptBA15*) is completed in 4 7 min,

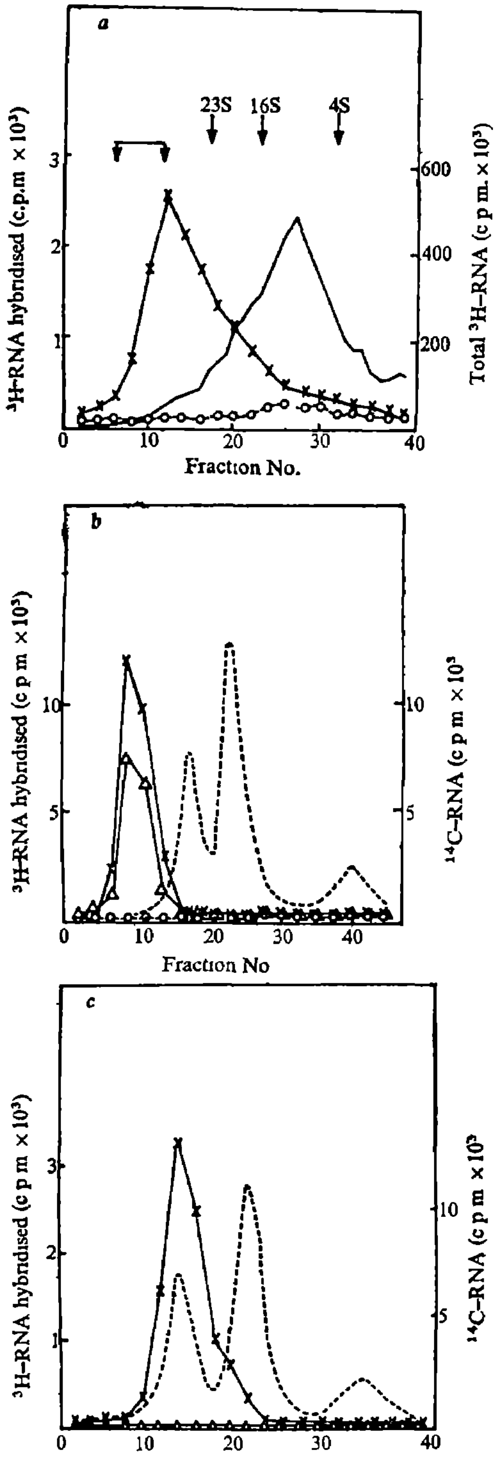

Fig. 2 Sedimentation (*a*) and resedimentation (*b* and *c*) profiles of *trp*mRNA of the mutants *trp*D9 and SO-584 *a*, The procedures used for pulse labelling, killing the cells and isolating ^{3}H-RNA are described in the legend to Table 1 Cells were labelled under derepression conditions (that is in the absence of tryptophan) The purified ^{3}H-RNA preparation (300–500 μg RNA, 4×10^6 c p m) was sedimented on a 5–30% linear sucrose gradient in 0 01 M Tris-HCl buffer (*p*H 7 3) and 0 05M KCl for 6 h at 36,000 r p m in a SW39 swinging bucket rotor of a L2-65B Spinco refrigerated ultracentrifuge Fractions of 10-16 drops were collected, and after adjusting the concentration of KCl to 0 5M, two neighbouring RNA fractions were pooled and 1/5 of each pooled fraction used for determination of the A_{260}, total ^{3}H-RNA counts and the hybridisation assay while the remainder was kept for resedimentation The hybridisation procedure is described in the legend to Table 1 X, counts retained on *ptEA*-DNA filters O, counts retained on φ80-DNA filters, ——, counts incorporated into cold TCA—precipitable material (total RNA) *b*, The bottom fractions containing the heaviest *trp* mRNA species (indicated by arrows in *a*) were combined and mixed with ^{14}C-rRNA prepared as described below All the RNA was then precipitated with ethanol, resuspended in H_2O, layered on a 5–30% sucrose gradient and centrifuged as described above After centrifugation, two neighbouring fractions were combined and hybridised with various DNAs The ^{14}C-rRNA was prepared by growing a strain carrying a deletion of the entire *trp* operon (*trpPOABEDC167*) under repression conditions (see legend to Table 1) and labelling with 50 μC of ^{14}C-uridine (52 7 mCi mmol^{-1}) for 3 h at 37° C. The cells were collected, washed twice and resuspended in 10 ml of the same medium After culture for 3 further hours, they were killed and the ^{14}C-RNA extracted by the procedures described in the legend to Table 1 ×, counts retained on pt77-DNA filters, Δ, counts retained on *ptE*-DNA filters, O, counts retained on φ80-DNA filters, – – – –, ^{14}C-RNA *c*, Resedimentation profile of the heaviest *trp* mRNA species of the mutant SO-584 (*trpθAB-673*) X, counts retained on *pt77*-DNA filters, minus counts retained on φ80-DNA filters, Δ, counts retained on *ptE*-DNA filters, minus counts retained on φ80-DNA filters; – – – –, ^{14}C-RNA.

ın good agreement with the results in Fig 3 (column 6 ın Table 2) The results obtained with the multisite mutants are ın marked contrast to those obtained with the control and clearly support the ıdea that they carry deletions ın the early portion of the *trp* operon The relative sizes of these deletions are ın good agreement with those indicated by the genetic data

If these deletions extend past *trpP1* and join the remainder of the *trp* operon to an outside promoter they should show no recombination ın crosses to the promoter mutation *trpP1443* (Fig 1) A full description of this mutation will be published separately (Angelosanto *et al* unpublished observations) Results of these crosses given ın Table 3 show that recombination occurs ın all cases and, as expected, is higher with deletion *trpAB688*, which does not extend into the operator, than with the others Of these *trpOA526* shows a higher recombination frequency than either of the two *trpOAB* deletions, which also make lower constitutive levels of both TSase and *trp* mRNA (Table 1) These results suggest that the *trpOAB* deletions extend partially into *trpP1* but *trpOA526* does not (Fig 1) A close examination of Table 1 reveals that while *trpOA526* makes between two and three times more *trp* mRNA than either *trpOAB673* or *trpOAB1349*, it synthesises between 20 and 30 times more TSase than the latter two deletion mutants Since *trpOA526* ends before *trpB* while the *trpOAB* deletions penetrate into *trpB*, the differences ın TSase levels observed these strains could be an indication that translation of the *trp* operon begins at initiators of different efficiencies ın these mutants

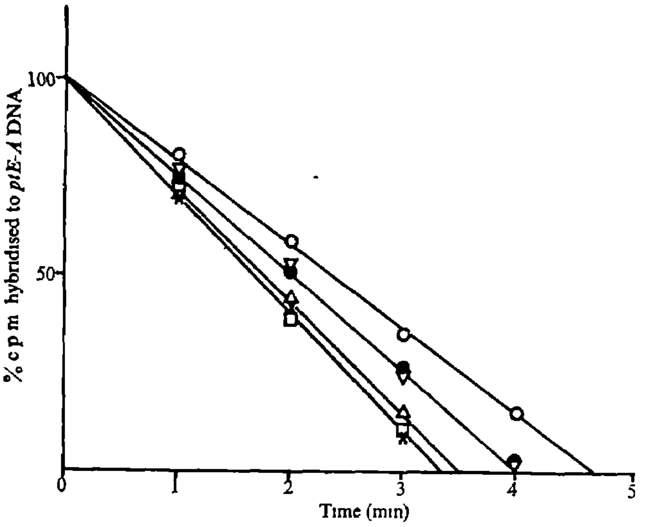

Fig. 3 Transcription of the *trp* operon after the addition of rifampicin Samples (4 ml) of overnight L broth cultures were used to inoculate 100 ml of fresh L broth, and the cultures incubated with aeration for 2 h at 37° C The cells were collected, washed with 0 03 M Tris-HCl buffer, *p*H 7 9, suspended ın 25 ml of the same buffer and incubated on a shaker for 2 min at 30° C At this point, 0 25 ml of a 0 1 M EDTA solution was added to the cell suspension, and incubation under the same conditions was continued for a further 2 minutes Pretreatment of the cells with EDTA ın Tris-HCl[28] made the cells susceptible to immediate inhibition by moderate concentrations (20 μg ml^{-1}) of rifampicin[29] At the end of this period, 75 ml of 4/3 strength Vogel and Bonner's minimal medium[16] supplemented with 1% glucose were added and incubation continued At the end of 10 5 min rifampicin (30 μg ml^{-1}) was added to the culture (time zero) Samples (10 ml) were removed at various times and pulse labelled for 30 s with ^{3}H-uridine (40 4 Ci mmol^{-1}) and killed as described ın the legend to Table 1 ^{3}H-RNA was isolated and hybridised with denatured DNA of *ptEA* transducing phage The percentage of c p m of ^{3}H-RNA hybridised is plotted at the midpoint of each pulse period after subtraction of the counts hybridised to φ80 phage DNA O, *trpD9*, ●, *trpOA526*, X, *trpOAB1439*, Δ, *OAB673*, ▽, *trp AB674*, ■, *trpAB688*.

Table 3 Recombination frequencies observed ın crosses between the promoter mutation *trpP*1443 and several deletions

Cross no	Recipient strain	Genotype	Frequency of recombinants 10^{-5}
1	SO-160	*trpOA*526	26
2	SO-441	*trpOAB*1349	3
3	SO-584	*trpOAB*673	1
4	SO-645	*trpAB*688	460

The crosses were carried out by transduction using the mutant L7 of bacteriophage P22 (ref 24) To increase recombination frequencies ın crosses 1, 2 and 3 the phage lysates were irradiated for 160 s with a GE germicidal bulb (15 W, polished reflector) at a distance of 60 cm These were optimal conditions for recombination, since phage lysates so treated showed a five-fold increase ın recombination frequency over unirradiated phage[25] In all crosses *trpP1443* was the donor and can grow slowly on minimal medium by forming pinpoint colonies after 48 h of incubation at 37° C Thus recombinants carrying *trpP1443* can be recognised by their typical phenotype The frequency of recombination is expressed as the number of prototrophs over total number of recombinants (prototroph + mutant) recovered In all cases, control platings of uninfected bacteria as well as phage lysates were carried out

All the results we have presented consistently indicate that, ın the *trp* operon of *S typhimurium*, the *trp* operator (*trpO*) and the trp promoter (*trpP1*) are essentially separable and arranged ın the order *P1-O* ın relation to the first structural gene *trpA* Brammar *et al* (personal communication) have reached the same conclusion for the *trp* operon of *E coli* Our data also show that deletion of most or part of *trpA* does not alter the regulation of the *trp* operon (that is *trpAB688* and *trpAB674*) but this is altered if the deletion includes *trpO* This gives further support to earlier findings[5,13,14] that the first structural gene of the *trp* operon does not play an essential role ın the regulation of the expression of the *trp* operon

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letters to nature

Observation of the 3_{12}–3_{13} transition of interstellar formaldehyde

Although several transitions of formaldehyde have been observed in interstellar space[1–7], there has been only one prior report of an observation of the 3_{12}–3_{13} transition[3] An energy level diagram of the lower rotational levels indicating the transitions that have been detected is given in Fig 1 The $\Delta J=1$ transitions are seen in emission The $\Delta J=0$ transitions are seen in absorption and emission[7–9] with a trend for the higher J transitions to be seen in emission (additional confirmation of this is provided by the unpublished work of B Turner who has seen the 4_{13}–4_{14} transition in emission) Here we report emission from the 3_{12}–3_{13} transition at 28 975 GHz in Sagittarius A (Sgr A)

Observations were made with the 4 57-m Cassegrain paraboloid at the University of British Columbia The receiver was operated in a beam-switched mode with the half-power beamwidth being 10′ The first stage of the receiver was a balanced mixer with a double sideband system temperature of 700 K In this work, however, losses from the switch, which was not operating at its centre frequency, and ground radiation degraded the system temperature by a factor of two Calibrations of antenna temperature were made using a gas discharge tube which in turn was calibrated using thermal sources at liquid nitrogen and ambient temperature Spectral line data were taken with a filter spectrometer having 32 contiguous channels each of 1 MHz bandwidth

The measured spectrum (Fig 2) is the average of two equal amounts of data one of which had been shifted in frequency by 3 MHz The data were obtained over seven nights of clear weather and represent 6 93 h of integration on Sgr A Also shown for comparison is an 'off-source' spectrum representing 6 58 h of integration The off-source data were obtained in exactly the same manner as the on-source data, except that the feed horns were covered by absorbing material because of poor weather The much larger noise on the formaldehyde spectrum is presumably due to fluctuations in atmospheric emission at the large zenith angle (~ 80°) at which we observe Sgr A The result we claim is an emission feature with a maximum antenna temperature of ~ 0 5 K centred at 0 km s^{-1} and extending about ± 30 km s^{-1} The significance of the result is 3 σ and so it should be treated with caution until confirmed by other observers The feature at −82 km s^{-1}, although almost as significant as the broad feature centred at 0 km s^{-1}, is dependent upon a single datum point and is probably a spurious signal The probability of this happening in a single channel is much higher than in five adjacent channels

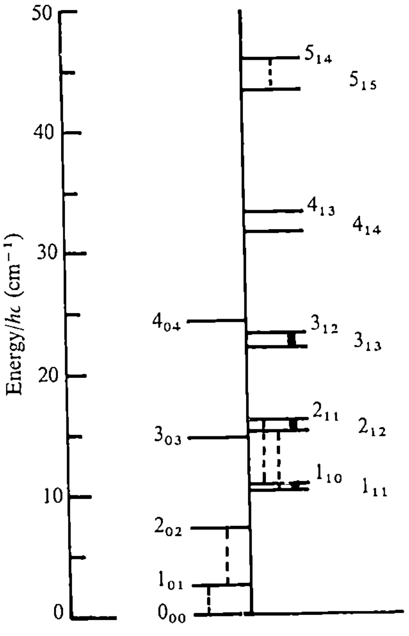

Fig 1 Observed transitions of H_2CO are indicated in the energy level diagram Transitions observed in emission are indicated by dashed lines while those observed in absorption are indicated by full lines

The velocity range covered by the emission line is somewhat different from the 6 cm formaldehyde absorption profile in Sgr A (ref 10) where the predominant absorption feature occurs over the range +20 to +65 km s^{-1} Other molecules such as NH_3 (ref 11), Xogen (ref 12), CO (ref 13) and CH_3 OH (ref 14) also give emission at positive velocities The 6 cm formaldehyde absorption profile, however, does have one component at 0 km s^{-1} but this is narrower than our emission result There is also agreement in velocity with the emission and absorption spectrum of HI (refs 15, 16) These agreements in velocity suggest a physical association of the emitting clouds

There are now nine different transitions that have been observed from $H_2{}^{12}C^{16}O$, but most of these have not been studied very extensively The 3_{12}–3_{13} line should be confirmed and more data should be obtained on the sizes and distribution of the $\Delta J=0$ emitting regions in order to have a clearer understanding of the factors involved in the excitation process

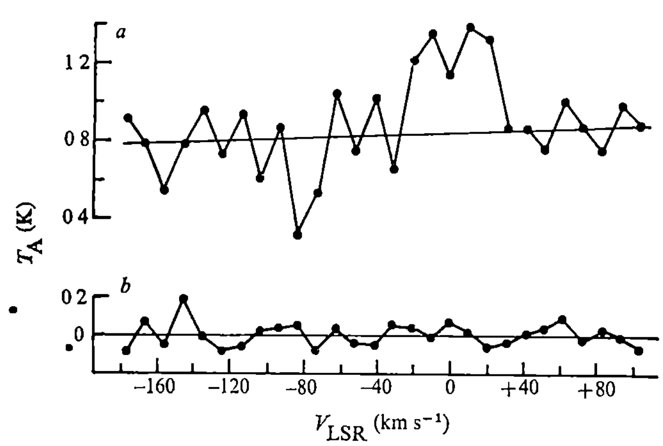

Fig. 2 Line profile of formaldehyde and control spectrum a, $J_k=3_{12}-3_{13}$, time 6 93 h, b, off-source, time 6 58 h

In the course of these observations we also searched for the $J_k=1_0-0_1$ transition of SO at 30 001 GHz in the molecular source in the Orion Nebula and obtained an upper limit to our antenna temperature of 0 2 K The 4_3-3_2 and 3_2-2_1 transitions have previously been observed[17], and if the column densities were the same at 30 GHz as at 99 3 GHz, we would expect an antenna temperature of 0 6–1 2 K Our upper limit implies an upper limit to the angular size of the SO emitting region ~ 5′ which is comparable to the sizes of the HII region and the H_2CO 2 mm emitting region

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Evolution of the Moon's orbit and the origin of life

PERIODICITIES in fossil corals may be used to determine the number of days in a year[1], both daily and seasonal variations are visible in the growth patterns Scrutton[2] used similar data to determine the number of days in a lunar month, monthly and fortnightly periodicities are present because of tides The available data[3–6] through the Palaeozoic are given in Fig 1*a* and *b* We conclude, from studies of living crustaceans and recent fossils, that the growth patterns are sensitive to the solar day So, the data represent the number of solar days in a year and in a lunar month, the present values are 365 25 and 29 53 d respectively Panella[3] has also used periodicities in stromatolites to determine the number of solar days in a lunar month during the Precambrian His results are given in Fig 1*c*

Palaeontological data can be used to determine the past history of the lunar orbit[7–9] Assuming conservation of angular momentum in the Earth–Moon system the length

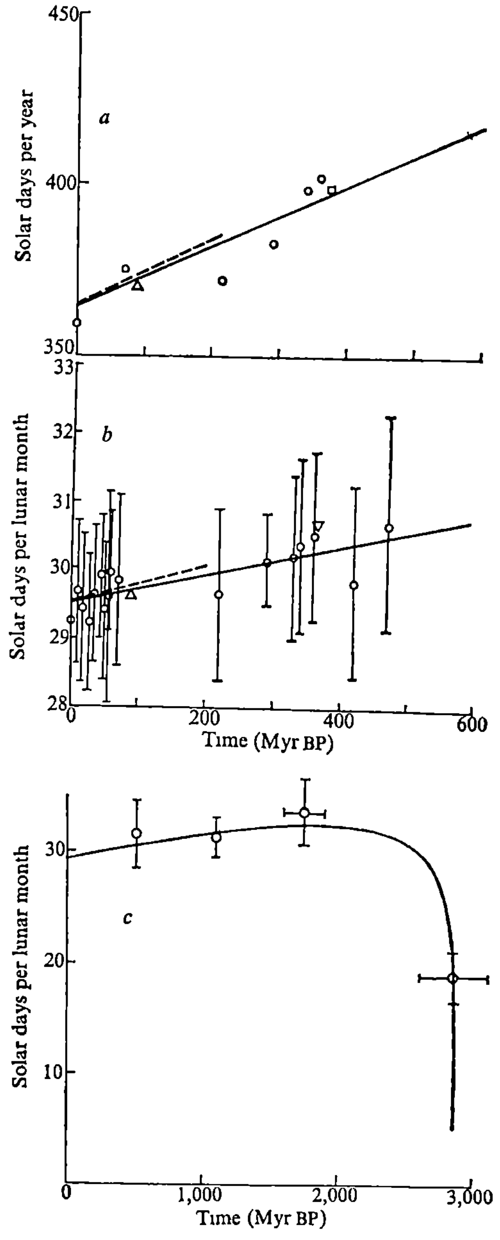

Fig 1 *a*, Data on the number of solar days in a year from molluscan shells is compared with the dependence we predicted and the value deduced from satellite observations (Newton[11]) *b*, Data on the number of solar days in a lunar month as in *a* Error bars are standard deviations of the data *c*, Data on the number of solar days in a lunar month from stromatolites (Pannella[3]) compared with the predicted dependence from our work The error bars on the counts are the standard deviations of the data, the error bars on the time are the stated errors on the dating of the fossils ○, Ref 3, □, ref 4, △ 5, ref 6, — — —, ref 11, ——, our results of the solar day and lunar month can be related, it is also assumed that the length of the year remains constant A lunar month cannot have more than 32 5 solar days (refs 8, 10) Because the Precambrian stromatolite data given by Panella[3] yield only monthly data, he could not show that the number of days in a month was not a multiple of the values given in Fig 1*c* But, the dynamical constraint on the number of days in a month places an upper limit which precludes, within the standard deviation of his data, an alternative interpretation of the data

With an assumption on the rate of tidal dissipation, the time evolution of the lunar orbit and length of day can be determined If the dissipation is proportional to the tidal force the rate of energy dissipation is proportional to the sixth power of the Earth–Moon separation Varying the single unknown parameter, the constant of proportionality between the tidal force and the tidal dissipation, the best fit to the data is given by the solid lines in Fig 1*a*, *b* and *c* In determining these curves the Bulawayan data at 2,850 Myr BP are critically important Without these data a close approach could not be predicted The counts of the Bulawayan growth patterns favour the 18 7 d month since there is a strong peak at 9 d corresponding to the fortnightly tides The alternative interpretation of a 37 4 d month falls outside the allowed maximum number of solar days in a lunar month even when the full standard deviation of the data is subtracted

The predicted value for the present tidal dissipation is $2\ 58\times10^{19}$ erg s^{-1} The corresponding value for the present acceleration of the moon is $n=-19\ 3$ and the value for the present acceleration of the Earth's rotation is $\omega=-934$ s $(100\ yr)^{-2}$ These values are in excellent agreement with the value for the acceleration of the Moon obtained from satellite observations[11] Newton's preferred value for the present lunar acceleration is[11] $n=-22\ 4$ s $(100\ yr)^{-2}$ and the corresponding changes in the number of solar days in a year and in a lunar month are given in Fig 1*a* and *b* Jeffreys[12] has reviewed the measurements of lunar acceleration and acceleration of the Earth's rotation and favours the value given by Newton The assumption that the dependence of the tidal dissipation on the tidal force is independent of time on the time scale of 10^9 yr certainly cannot be justified in detail But the resulting calculations are consistent with observation In Fig 2 the dependence of the Earth–Moon separation on time is given It seems that the Moon approached the Earth 2,850±250 Myr BP If the calculations of Gerstenkorn[13] are accepted this close approach was near the Roche limit for tidal catastrophe In any case, a near approach would result in very large tides and large energy dissipation Tidal heating could possibly have vaporised the oceans This has both geological and biological implications

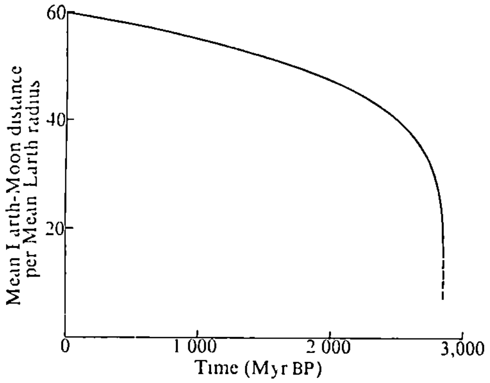

Fig 2 Predicted dependence of the Earth–Moon separation on time

There is evidence in the geological record[14] that a pulse of high temperature volcanism occurred about 2,800 Myr BP Many of the high temperature intrusions of ultrabasic rocks are dated at this time Such intrusions could be the result of tidal heating near the surface

The implied catastrophe roughly coincides with the first records of life The Onverwacht Series of southern Africa, which contains the first cell-like objects[15–18] is, contrary to earlier estimates[19], at least 3,000 Myr old (ref 20) and at most 3,200 to 3,300 Myr old (refs 21, 22) The oldest extensive Banded Iron Formation, one indirect indication of life[23], has been reliably dated[24] at no more than 3,000 Myr BP The earliest conclusive evidence for life is, in fact, the Bulawayan stromatolites[25–27] Though stromatolites generally form in warm waters, it has been suggested that many Precambrian-type stromatolites formed in scalding waters[28,29], which is consistent with the idea of thermal catastrophe It seems within the realm of possibility that a global thermal event might have been involved in the origin of life On a grand scale, one such event would resemble experiments performed by Fox[30,31] in which heating and cooling of 'organic soup' leads to the formation of remarkably cell-like coacervates

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Relative abundances of positrons and antiprotons in the primary cosmic ray flux

SHEN and Berkey[1] calculated the ratio of antiprotons and positrons to be expected in the primary cosmic rays assuming that both components are the end products of proton–proton interactions, and pointed out that the observations of the positron spectrum would permit us to deduce the antiproton energy spectrum Observations on the ratio e$^+$/(e$^+$ + e$^-$) have been made[2] in the 5–50 GeV range, and we have used the results to put an upper limit on $\bar{p}$/p at various energies

Our calculations on the expected antiprotons-positron ratio differ in one significant respect from those of Shen and Berkey[1] we have detailed measurements of the cross section of antiproton production up to intersecting storage ring energies That permitted us to calculate the e$^+$/$\bar{p}$ ratio quite reliably

We have plotted the latest $\bar{p}$ and π^+ production cross-sections (Fig 1) They were deduced from the work of Antinucci *et al*[3] and Ramaty and Lingenfelter[4]

It has been shown[5 6] that $\langle E_{\bar{p}}\rangle/E_p$, the ratio of the mean energy of antiprotons, $\langle E_p\rangle$, to incident-proton energy, E_p is approximately 9, independent of the energy of the incident protons

This differs significantly from the results of Shen and Berkey[1] who found this ratio to vary as $E_p{}^{1/4}$ For the purpose of this calculation, we can summarise the results of the kinematics

$$\left.\begin{array}{l}\sigma_{\bar{p}}(E_p) \simeq 9\ 3 \times 10^{-3}(E_p - 10\ 8)\ \mathrm{mb} \\ \langle E_{\bar{p}}\rangle \simeq 9\ E_p\ \mathrm{GeV}\end{array}\right\} 20 \lesssim E_p \leq 550\ \mathrm{GeV} \tag{1}$$

$$\left.\begin{array}{l}\sigma\pi^+ (E_p) = 27\ 7\ E_p{}^{1/4}\ \mathrm{mb} \\ \langle E_{e^+}\rangle = 0\ 175\ E_p{}^{3/4}\ \mathrm{GeV}\end{array}\right\} 50 \lesssim E_p \lesssim 1{,}500\ \mathrm{GeV} \tag{2}$$

Following a procedure analogous to that of Shen and Berkey[1] we found that the ratio of the production spectrum of positrons to antiprotons can be written as

$$\frac{Q_{e^+}(E)}{Q_{\bar{p}}\ (E)} = \frac{6\ 176 \times 10^{-2}\ E^{-0\ 586}}{2} \times \frac{\sigma_{\pi^+}[(22\ 86E^{4/3})]}{\sigma_{\bar{p}}(9E)} \tag{3}$$

which gives rise to values for the ratio e$^+$/$\bar{p}$ of 4 at 5 GeV and 1 56 at 10 GeV These values depend on the spectral index of the differential proton energy spectrum only, taken to be 1 9 × $10^4 E^{-2\ 75}$m^{-2} sr^{-1} s^{-1} GeV^{-1} (refs 7 and 8) The factor of two in the denominator comes from the decay of antineutrons in space

The equilibrium value of e$^+$/$\bar{p}$ will depend on the actual model of propagation and would tend to lower somewhat the ratio e$^+$/$\bar{p}$ Similarly, if the leakage mean free path is energy dependent this ratio will again be lowered We will ignore these effects for the present and assume that the production and equilibrium values are the same They will change the ratio by a factor of two or less

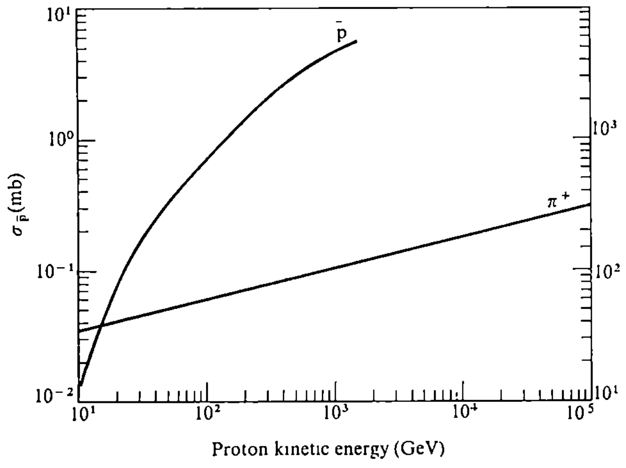

Fig. 1 The production cross sections of π^+ (scaled down by a factor of 1 5) and $\bar{p}$, as a function of proton kinetic energy in proton–proton collisions

We interpret the results of Buffington *et al*[2] as indicating an upper limit of 10% on the ratio e$^+$/(e$^+$ + e$^-$) in order to obtain an upper limit on p/p That gives an upper limit on the e$^+$ energy spectrum when combined with the measurements of (e$^+$ + e$^-$) as summarised by Muller and Meyer[9] Thus,

$$\mathrm{p/p} \lesssim 1\ 8 \times 10^{-4} \text{ at 5 GeV}$$
$$\lesssim 5\ 7 \times 10^{-4} \text{ at 10 GeV}$$

which, even after allowing for propagation uncertainties, is about an order of magnitude lower than the value of 6 × 10^{-3} obtained by Bogomolov *et al*[10] in a larger GeV range

As the e$^+$/p ratio produced in nuclear reactions is very well known, measurements of e$^+$/p (in conjunction with knowledge of the proton flux itself) depend only on the amount of e$^+$ from cosmic ray sources and on the energy dependence of the storage time of primary protons in the galaxy Comparison of e$^+$ and p fluxes would be extremely valuable in the understanding of cosmic ray production and propagation

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Will there be a large earthquake in central California during the next two decades?

P-VELOCITY decreases before earthquakes[1–4] can be observed by monitoring the average P-residual at seismic stations near the focus[5–7] The P-residual is the difference between the observed travel time from an epicentre to a station and that computed theoretically on the basis of some Earth model and a least squares estimate of the earthquake location and origin time If the P-velocity in the crust underneath a seismic station is decreased temporarily, each seismic wave will arrive somewhat later during the premonitory period Before three thrust[5,6,18] and one strike-slip event[7] the delay was found to be approximately 0 4 s

Here I compare the average teleseismic P-residuals of four stations located near the San Andreas fault (Berkeley, BKS, Mt Hamilton, MHC, Priest, PRI, and Pasadena, PAS) to those of three stations to the East of it (Mineral, MIN, Jamestown, JAS, and, Golden, GOL) (Fig 1) Since our interest is focused on the detection of precursor anomalies lasting several years (earthquake magnitudes ⩾6 5) Fig 2 shows annual mean residuals The standard deviations of the annual means are approximately 0 1 s for 1961–63, and 0 07 s to 0 04 s since then, shown by error bars in the figures.

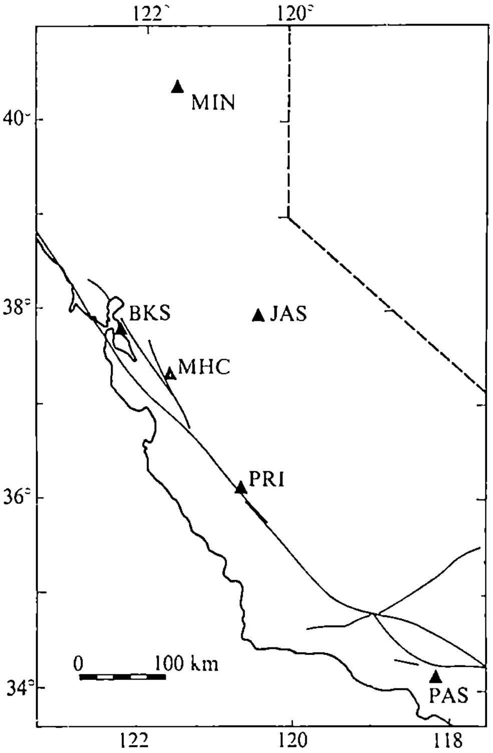

Fig. 1 Map of California showing the locations of seismic stations with respect to the San Andreas fault The station Golden is located in Colorado at 39° 42′N and 105° 22′W

The constancy of the average P-residuals in Fig 2 is surprising compared with other data[5-7] where fluctuations of 0 15 s are quite common and where precursor anomalies have amplitudes of 0 4 s Only stations PRI and PAS show disturbances of more than 0 2 s from the long term mean Both disturbances accompanied earthquakes near those stations All curves except that for MIN show an increase of approximately 0 2 s in the mean annual P-residual in 1969 Clearly the increase in 1969 was due to changes in the earthquake locating procedure, since a similar change occurs at stations in other parts of the world Therefore I assume that the stations GOL, JAS and MIN are in a different tectonic region than the San Andreas stations and thus these three observatories can be used as control stations

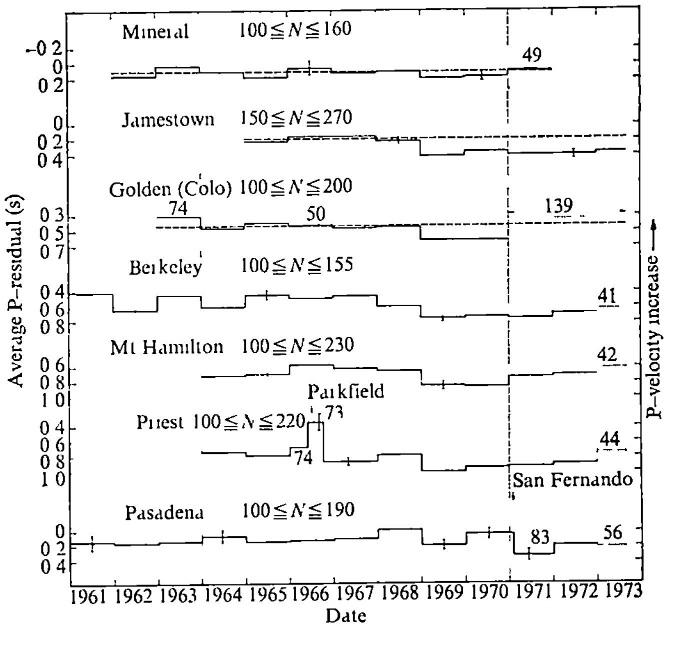

Fig 2 Mean annual teleseismic P-residuals from 3 reference (top) and 4 tectonic (bottom) stations Dashed lines, long term average through 1968 Arrows indicate time of occurrence of local earthquakes Vertical dashed lines separate different data sets The data for the years 1961–1963 are from ref 8, those for 1964–70, from ref 9, and those for 1971–73, from ref 10 All residuals used were from earthquakes with depths ⩾ 100 km, located at distances ⩾ 15° whose hypocentres were determined on the basis of more than 25 readings and with absolute values smaller than 3 s.

Errors related to the seismic source, the teleseismic ray path, or the data procurement procedure are reduced by taking the difference between the average residuals at different stations (for details see ref 6) Each tectonic station was compared simultaneously to as many of the three control stations as were operating each year For this purpose a correction factor was computed which was the average of the control station deviations from their respective long term means The average residual minus the correction factor is shown in Fig 3

The stations BKS, MHC, PRI and JAS are operated on a common time base by the University of California, Berkeley, and the same observer reads the arrival times at all stations For these stations the comparison with the control station JAS will therefore be especially effective in reducing all errors except the effects of changes in the local crust and the unlikely possibility of changes in the instrument characteristics The corrected P-residuals shown in Fig 3 are very stable with deviations from the respective long term means of less than 0 13 s for all years, except at the times of the Parkfield and San Fernando earthquakes (The averages for 1973 do not contain enough data points to be considered reliable)

The decrease of the average residual at PAS before the San Fernando earthquake suggests that PAS was located just outside the dilatant region which preceded this earthquake[3], and that the crustal P-velocity under PAS increased temporarily This may have been a consequence of stresses generated by the dilatancy closing rock pores at the periphery After the earthquake a somewhat lower than average velocity is indicated by the P-residuals This may have been due to partial dilatancy outside the source region caused by the redistribution of stress associated with the earthquake Such an effect is expected from laboratory experiments[13] and was observed for the 1966 Gisborne earthquake in New Zealand[6] The results presented for PAS are in agreement with the constancy of the P-velocity as determined from records of quarry blasts[16] and individual teleseisms[17]

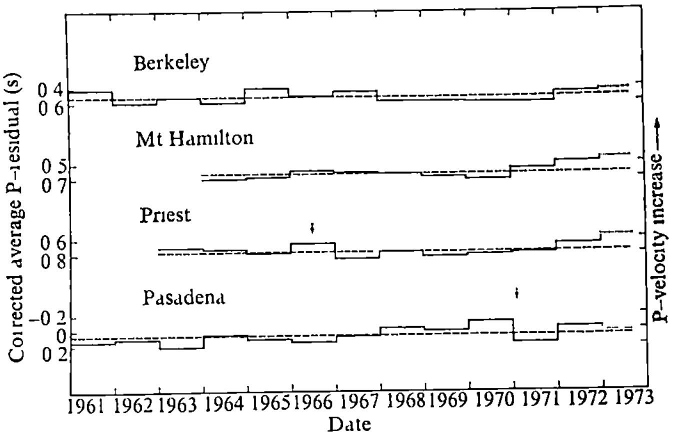

Fig. 3 Corrected annual mean residuals for 4 tectonic stations

From Fig 3 it is clear that, except for the minor perturbations discussed above, there is no significant change in P-velocity in a vertical direction beneath any of these stations. McEvilly and Johnson[14] concluded from explosion studies that there was no variation in both the P and S-velocities in a horizontal direction in central California. In addition they found v_p/v_s to be normal.

The absence of a P-residual anomaly in central California can be interpreted to mean that strike-slip tectonism does not produce significant precursory changes in the seismic velocity. But it seems that the P-residuals increased before the strike-slip earthquakes at Sitka, 1972 (ref. 7), and at Bear Valley, 1972 (ref. 15). These two observations suggest that the above interpretation of the data may not be correct. Since magnitude 7 and 8 earthquakes are expected to be preceded by anomalies lasting 7 and 25 years respectively[3,12], the data presented here and in ref. 14 suggest that no earthquake of $M>7$ and no earthquake of $M\approx 8$ will occur between San Francisco and Parkfield, California during the next 7 and 25 yr, respectively.

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Is Kakangari a unique chondrite?

THE Kakangari meteorite, possibly a unique member of a distinct chemical class of chondrite, fell in India in 1890. Two stones were seen to fall, and though one was soon destroyed the bulk of the other, about 310 g, is preserved in Calcutta and London[1,2]. Mason and Wiik[3] concluded that Kakangari is probably a carbonaceous chondrite. Subsequent improved understanding of chemical fractionation between chondritic groups (see ref. 4) enables us to show, however, that their analysis in fact indicates that Kakangari is similar in bulk composition to the ordinary chondrites, apart from its high S content (Table 1). In this last respect it resembles carbonaceous or enstatite chondrites. The FeO × 100/(FeO + MgO) molecular ratio, 13.7, lies between that of H group (bronzite) chondrites and enstatite chondrites. That is confirmed by our microprobe analyses discussed here. The mineralogical data are based on preliminary microprobe analyses on a thin section cut from a British Museum (Natural History) specimen (BM 69062).

Table 1 Composition of chondrites*

	C†	O	K	E
Mg	10,500	9,500	9,900	7,500
Ca	730	480	489	355
Al	850	610	726	480
S	5,000–1,200	1,000	2,800	3,000–1,500‡

*Atoms per 10^4 Si atom.
†C, carbonaceous chondrites, O, ordinary chondrites, K, Kakangari, E, enstatite chondrites.
‡The high figures are for C1 and E3, 4, respectively. Kakangari data from ref. 3, the remainder from ref. 12.

Olivine is apparently less abundant than pyroxene. Analyses of 26 grains from 15 locations show the compositional range to be $Fa_{3-9.5}$, the grains have less than 0.1% CaO (weight) and in this respect are similar to the olivines of ordinary chondrites[5] and distinct from those of carbonaceous chondrites[6]. Mason[3] used a diffractometric method to determine the composition of Kakangari olivine and from the skewed peak he obtained, suggested that the olivine is dominantly forsteritic with some scatter towards more iron-rich compositions, perhaps Fa_{25}. Our microprobe work does not substantiate this and an X-ray powder photograph shows that the groundmass is dominantly enstatite with some troilite. That eliminates the groundmass as a possible source of olivine, as in type III carbonaceous chondrites. In any case, the compositional distribution of Kakangari olivines is more limited than in type III carbonaceous chondrites[7], it lacks a peak at Fa_1 or the more Fe-rich grains to Fa_{50} or more. The mean olivine composition in Kakangari—$Fa_{4.9}$—is, however, close to that of the carbonaceous chondrite Vigarano, $Fa_{5.6}$ (ref. 7).

Fifteen analyses of pyroxene from 11 locations indicate that the compositional range is $Fs_{4.6-14.5}$, with the wollastonite component from $Wo_{0.2-6.6}$, grains from 7 of the 11 locations (a location is a chondrule or grain in matrix) were in the range $Fs_{4.5-5.5}$. The mean pyroxene composition is $Fs_{6.9}$ and is similar to that of carbonaceous chondrites (ref. 7).

Troilite is ubiquitous, occurring as grains and veins in chondrules, and as grains or fine grained material in the groundmass. Its Ni content is less than 0.2% by weight, but grains in the groundmass have 0.19 ± 0.05% Cr (weight) and a grain in one chondrule has 0.43 ± 0.05%. The Ti content is less than 0.05%. Kamacite occurs within chondrules, as rims ('armour') to chondrules, and as grains in the matrix. Ni and Co contents are close to 6% and 0.7% (weight), respectively. In our cursory examination, taenite was not found in the matrix, but it commonly occurs as grains in chondrules. Most grains have a fairly uniform Ni content in the range 29–32%, but one zoned grain has a core with 26% increasing to 31% or more at the rim, indicating some attempt at equilibration during falling temperature[8]. Taenite has a Co content of about 0.31%, and will be studied in greater detail.

This meteorite has a state of reduction between that of the H group (bronzite) chondrites and enstatite chondrites. In ordinary chondrites, Cr is lithophile, the sulphide containing not more than a few hundred p.p.m. of this element (ref. 9, and C. J. Elliott, personal communication) but in the enstatite chondrites, the troilite has about 1% Cr by weight and the remainder of this element occurs as the sulphide, daubreelite[10]. In enstatite chondrites, Ti, is also largely chalcophile, the bulk, about 75%, occurring as a minor constituent of troilite[11]. The presence of significant Cr and absence of Ti in the Kakangari troilite thus indicate a state of reduction between that of enstatite and ordinary chondrites. This conclusion is also supported by the oxidised Fe content of the olivine and pyroxene; the enstatite chondrites have almost no Fe in the silicate[10] whereas ordinary chondrites have olivines and pyroxenes in the range Fa_{16-33} and Fs_{14-25}, respectively (6). The mineral chemistry thus supports our interpretation of Mason and Wiik's[3] bulk chemical analysis as indicating that Kakangari represents a class of chondrite previously unrecognised.

Because it is apparently unique, the limits (if any) of the chemical properties diagnostic of its class cannot yet be determined. Although closer to the L group of ordinary chondrites with regard to its low (22.47%) total Fe content, its oxidation state indicates a closer relationship with the H group. It is chemically distinct from enstatite and carbonaceous groups. Perhaps it is best to consider this stone as representative of a K group of chondrites, the diagnostic properties of which are undefined.

A. W. R. and J. C. Bevan, and R. F. Symes, are thanked for sample preparation or help with microprobe analyses. E. E. Fejer arranged the X-ray powder photograph.

Note added in proof: We now realise that Kakangari may be chemically related to the recrystallised chondrite Mount Morris (Wisconsin), to the silicate portion of the stony-iron Winona, and to chondritic xenoliths in the achondrite Cumberland Falls; we have begun to investigate this.

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A Cretaceous guyot in the Rockall Trough

THE Anton Dohrn seamount[1,2] is situated in the central part of the Rockall Trough (Fig. 1). The feature is of some interest in view of its relatively isolated position in the trough and because it has an almost flat summit, a rare characteristic for a seamount in the North Atlantic. Because it has received little attention since its early bathymetric survey, we took the opportunity of recording magnetic and seismic profiles over it and also carried out a preliminary sampling investigation in the area during a recent cruise of *RRS John Murray*. In this contribution we report some of our findings and discuss their significance in relation to the history of the region.

The seamount possesses a strong magnetic signature (Fig. 2) in marked contrast to the main part of the Rockall Trough which is magnetically quiet[5,6]. The high amplitude, short wavelength anomalies, together with the general shape of the seamount, are consistent with its being a volcanic feature. It is clearly different in composition and origin from the nonmagnetic Galicia Bank[7], another flat-topped seamount which is situated close to the European continental margin near 43°N. Seismic profile A_1–B_1 in Fig. 3 reveals the presence of a capping of sediments which, assuming an average sedimentary sound velocity of 2.0 km s^{-1}, reaches a thickness of over 100 m. Although the seismic evidence is not unequivocal, the contact between the sediments and the main volcanic pile appears to be defined by the reflector labelled X, the major break in slope on the seamount occurring where this horizon outcrops. A 200 kg haul of rocks obtained by dredging the steep slope just below the bench at which reflector X is exposed (site 4, Fig. 1.) supports this interpretation. Over 60% by weight of the material recovered consists of ahermatypic corals (mainly *Lophelia prolifera*) and not unexpectedly, exotic blocks of glacial origin. The remaining fraction is made up of basic lavas and tuffs, many being highly altered and very friable, which are considered to have come from outcrops situated along or close to the path taken by the dredge. The composition of the central portion of the largest block of lava, one weighing 38 kg, is given in Table 1. This is a basalt of alkaline affinities which is predominantly made up of plagioclase feldspar (An_{68}–An_{72}), together with augite and hornblende, the latter replacing the pyroxene. Unfortunately, the extent of the alteration makes it unsuitable for radiometric dating.

Even though the basic volcanics are far from fresh they have proved to be of considerable interest, because chalk was found adhering to several specimens. The chalk either occupies vesicles, as in the case of the 38 kg block, or it occurs as a matrix between highly altered, angular and sometimes vesicular clasts of basalt (see Fig. 4). Despite its intimate association with the lava, the sediment shows no evidence of baking. The chalk contains abundant nannofossils whose preservation is, unfortunately, somewhat marred by calcite overgrowths. Nannofossil assemblages are assigned to the *Nephrolithus frequens* Zone of Cepek and Hay[8] which Manivit[9] considers to be Upper Maestrichtian in age, correlating it with the *Globotruncana contusa* foraminiferal zone. The following species were recorded: *Nephrolithus frequens, Lithraphidites grosspectinatus, L. carniolensis, L. quadratus, Tetralithus murus, T. quadratus, Markalius astroporus, Lucianorhabdus cayeuxi, Ceratolithoides kamptneri, Cylindralithus serratus, Rhabdolithina splendens, Reinhardites anthophorus, Microrhabdulus stradneri, Cretarhabdus decorus.* The scarcity of members of *Lithraphidites quadratus* and *Nephrolithus frequens* together with the absence of pentaliths suggests an open ocean assemblage[10,11].

The time of formation of the Anton Dohrn seamount cannot be determined with any certainty without more extensive sampling but the discovery of Maestrichtian chalk so closely associated with the volcanics points to an Upper Cretaceous age. The chalk may indeed be the earliest sediment to be deposited on the seamount. Evidently, the feature was not created at the time of the main separation of the Rockall Plateau from Europe because it is characterised by large magnetic anomalies whereas most of the Rockall Trough is magnetically quiet. The Cretacous age and the spatial relationship of the Anton Dohrn seamount with respect to the volcanic centres in north-east Ireland and Scotland make it difficult to envisage the feature as part of the scar trace of the mantle plume postulated by Duncan and others[12] to explain age variations in the Thulean province. It is, perhaps, more likely to be related to the lithospheric heating and general tensional conditions in the crust which immediately preceded the development of new ocean basins to the west and north of the Rockall microcontinent.

An appreciable proportion of the seamount was removed by erosion following the main volcanic activity, the even surface of reflector X probably being the level to which erosion extended. The central knolls (Fig. 1) are possibly remnants of resistant feeder channels within the volcano.

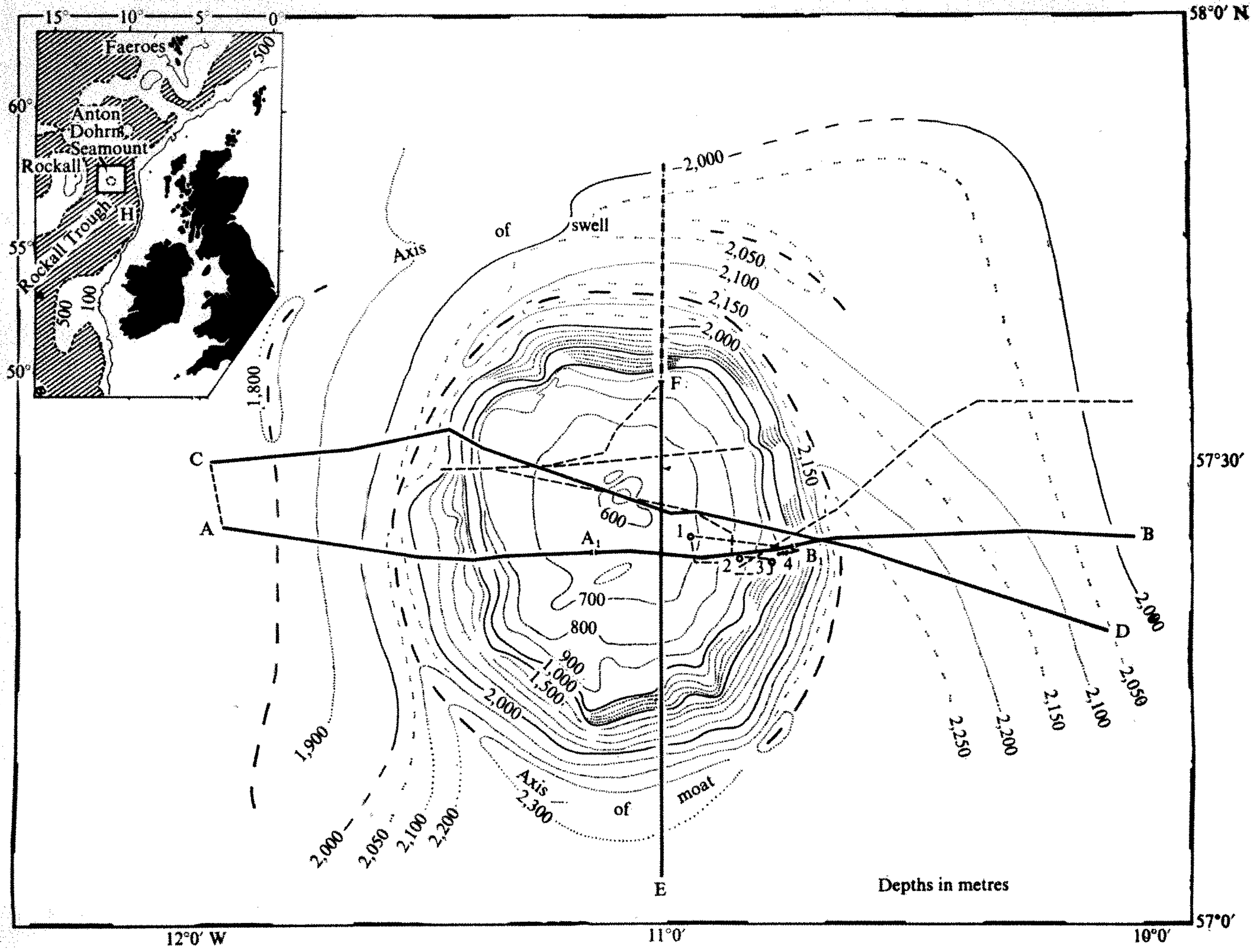

Fig. 1 Tracks of *RRS John Murray* shown on a bathymetric map of the Anton Dohrn seamount, taken from Dietrich and Ulrich[2]. Water depths are corrected for sound velocity variations in sea water using the tables of Matthews[3]. Profiles along portions of the track indicated by heavy lines are reproduced in Figs 2 and 3. Positions of core stations 1–3 and dredge site 4 on the eastern side of the seamount are indicated. The moat and swell around the seamount arise from non-uniform deposition of sediments controlled by the pattern of bottom currents[4]. H = Hebrides Terrace.

Similar features have also been recognised on some of the Pacific guyots[13]. A comparison of the shapes of volcanic islands and the Anton Dohrn seamount suggests that it originally stood at least 2 km above sea level. After the period of planation the volcano sank over 600 m. Its present, southeasterly tilt (Figs 2 and 3) may have been acquired at this time or it may reflect subsequent loading of the crust by thick sediments which airgun profiles indicate lie beneath the lower part of the continental slope between the seamount and the Hebrides Terrace (H in Fig. 1).

Coring was carried out upslope from the dredge site (stations 1–3; Fig. 1) to determine the time and rate of sinking of the seamount from the stratigraphy of the sediment layer above X. According to the seismic profiles, reflectors normally lying within the layer are exposed there. Unfortunately, the corer failed to penetrate a cover of stiff Glacial or post-Glacial shelly and silty clays so, at present, we can only assign a tentative date for the subsidence from what is known about vertical movements in the surrounding areas. In this region of the North Atlantic there is evidence for at least one major phase of regional subsidence in post-Cretaceous time. Seismic profiles from the continental margin west of Britain reveal a widespread unconformity, believed to be of early Tertiary age from a limited amount of stratigraphic data, which appears to have resulted from erosion followed by downwarping of the margin and outbuilding of the continental slope[14,15]. To the west of the Rockall Trough the sedimentary record of the Rockall Plateau provides convincing evidence of early Tertiary subsidence. The lithological variations at DSDP site 117 show that at least part of the plateau sank 600–700 m in late Palaeocene–early Eocene times at an average rate of 14 cm per 1,000 yr (ref. 16). Although the movements to the east and west of the Rockall Trough may not have been synchronous, there is general agreement that an important episode of subsidence took place between the late Cretaceous and Oligocene, possibly related to cooling of the lithosphere in this region following the birth of the Reykjanes Ridge and the early Tertiary igneous activity in the Rockall and Scottish areas. It seems probable that sinking of the Anton Dohrn seamount is yet another manifestation of these early Tertiary crustal adjust-

Table 1 Analysis of basalt dredged at site 4

	SiO_2	Al_2O_3	Fe_2O_3	FeO	MgO	CaO	Na_2O	K_2O	H_2O	TiO_2	P_2O_5	MnO	CO_2	Total
%	39.80	15.13	10.09	2.08	5.34	13.64	2.21	0.60	4.76	1.53	0.46	0.18	3.94	99.76

ments and is also an indicator of appreciable subsidence of much of the Rockall Trough at this time.

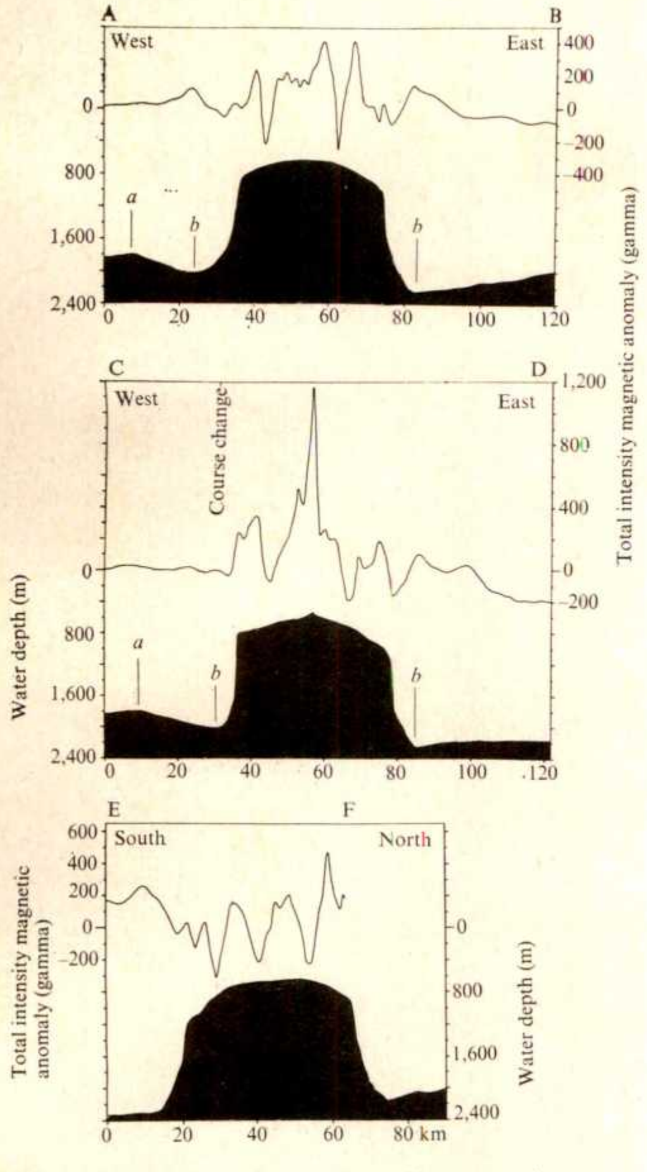

Fig. 2 Bathymetric and magnetic profiles across the Anton Dohrn seamount. The International Geomagnetic Reference Field has been subtracted from the total field observations to obtain the residual magnetic anomalies (1 nT = 1 gamma). *a*, Axis of swell; *n*, axis of moat.

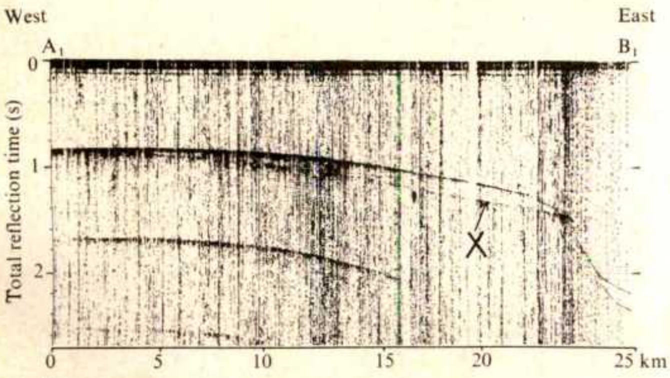

Fig. 3 Seismic reflection profile along line A_1–B_1 recorded using an airgun sound source.

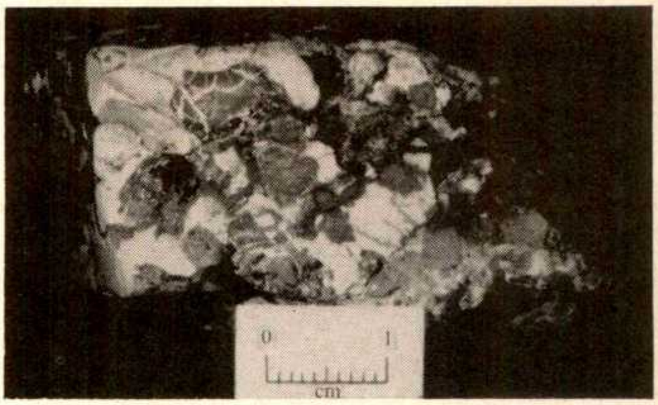

Fig. 4 A section showing the intimate association of the light- coloured Maestrichtian chalk and weathered volcanics.

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A proposed γ camera

ISOTOPES which emit γ rays are used for clinical diagnosis. This has led to the development of image forming systems which are based on detectors placed in the proximity of a radiating source, allowing abnormalities such as tumours and gross lesions to be detected.

As γ rays cannot be focused, the formation of an image depends upon an estimation of particle trajectories. Generally, this necessitates the elimination of all trajectories outside a narrow range of incidence angles. This has been achieved using lead collimators. Such systems give directional information but do not indicate the distance from the detector to the source point. Clearly only two-dimensional images can be obtained. Using a collimated scintillator and photomultiplier system severely limits the use of the cameras. We here outline a completely different approach to the principles of γ-ray imaging, which seems to offer considerable advantages over existing systems.

Scanners or cameras can be used. The scanner has a detector head consisting of a scintillation crystal, a photomultiplier and a lead diverging hole collimator. The complete image forming process can take up to 30 min. In camera systems the collimated detector must cover the entire region of investigation. Mechanical scanning is not used and the scintillation positions are detected using an array of photomultipliers. This principle is adopted in the widely used Anger system[1]. The interactions used in image formation are single absorption processes which result from the photoelectric effect.

In both scanner and camera imaging, speed can be increased with greater isotope activity. With a lead collimator only a small fraction of the incident radiation is useful. Also, with low energy isotopes (< 500 keV), there is considerable scattering within the examined region. There are thus inherent limitations in resolution and sensitivity.

The Compton effect is associated with multiple interactions. The position and intensity of these interactions define, within statistical limits, the trajectory of the incident photon. In principle this would allow projection back on to an image plane (Fig. 1). If the coordinates, $\mathbf{x}_1$ and $\mathbf{x}_2$, of the first two consecutive interactions are measured, and if the scattering angle, α, is known then the emission point must lie on the surface of a cone defined by all lines at angle α to the vector $\mathbf{x}_1 - \mathbf{x}_2$ through $\mathbf{x}_1$. The intersection of this cone with an image plane, generates an ellipse with parameters which are functions of $\mathbf{x}_1$, $\mathbf{x}_2$, and α. $\mathbf{x}_1$ and $\mathbf{x}_2$ can be measured directly using an array of detectors, which also give an indirect estimate of α from the energy loss, δE, at the first interaction:

$$\cos \alpha = (E - k\delta E) \,/\, (E - \delta E)$$

where E = initial source energy, and $K = 1 + (mc^2 \,/\, E)$, constant for a particular source.

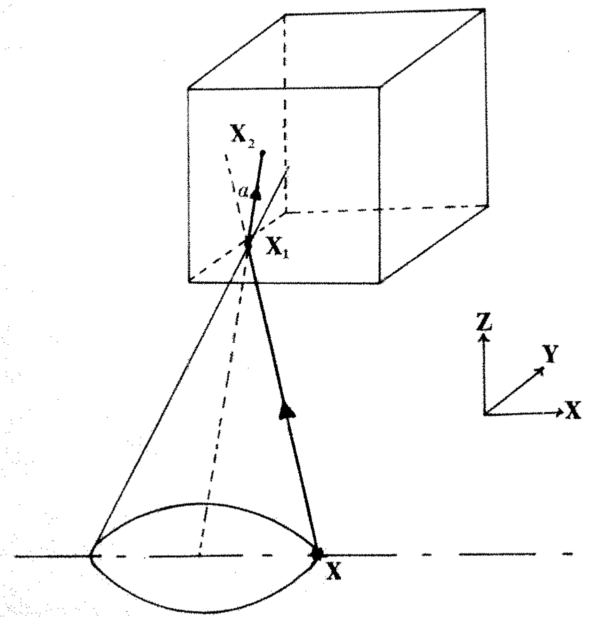

Fig. 1 The Compton γ camera principle.

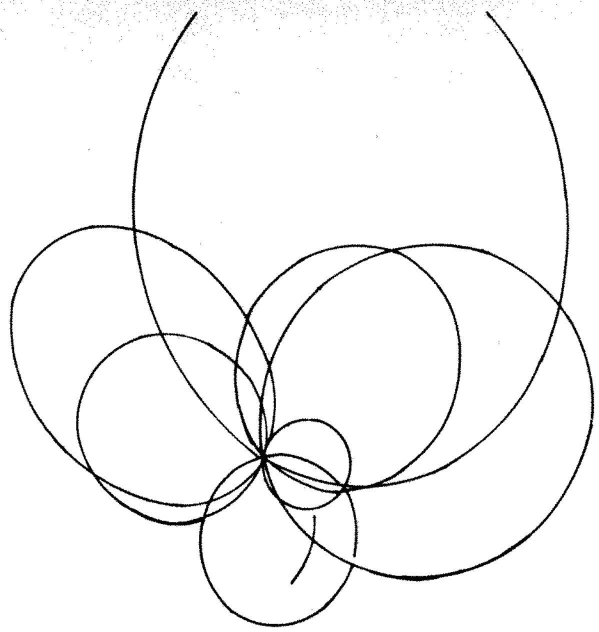

Fig. 2 Ellipses generated from a point source.

If a single point source of activity is considered, a succession of emissions will generate a number of ellipses which have a common point of intersection, thereby defining the image point (Fig. 2). The image of a radiating body consists of many small elements, each representing a small area of the source. The size of the elements is, therefore, chosen so that there are sufficient emissions from each to produce a statistically stable image. The simplest image which can be formed is by direct summation of all the ellipses produced. Generation of each ellipse involves the computation of coordinates, $\mathbf{x}$ from the equation relating the scalar product of the two vectors, $(\mathbf{x}_1 - \mathbf{x}_2)$ and $(\mathbf{x} - \mathbf{x}_1)$, to the scatter angle, α. For convenience this equation is written as

$$\cos \alpha = \overline{(\mathbf{x}_1 - \mathbf{x}_2)} \,.\, \overline{(\mathbf{x} - \mathbf{x}_1)} \qquad (2)$$

where a bar denotes a unit vector.

Vector $\mathbf{x}$ must also be constrained to lie in the image plane defined by

$$\mathbf{a} \,.\, \mathbf{x} = b \qquad (3)$$

The image plane is defined only by the parameters, a, and b, in equation (3), and so the choice of the depth and inclination of the plane is arbitrary. It is possible to project images on to a multitude of planes using the same set of measurements, and even to superimpose them to obtain three-dimensional images.

Figure 3 shows a schematic diagram of the proposed imaging system. There will be errors because of noise in the amplifiers, and because of quantisation and round-off into the digital processor. The basic source of error is, however, the detector, which is analogous to the lens in a normal optical system. As it is envisaged that the system will be able to cope with changes in the source distribution, the dynamic aspects of these errors are of considerable importance in relation to the design of the components.

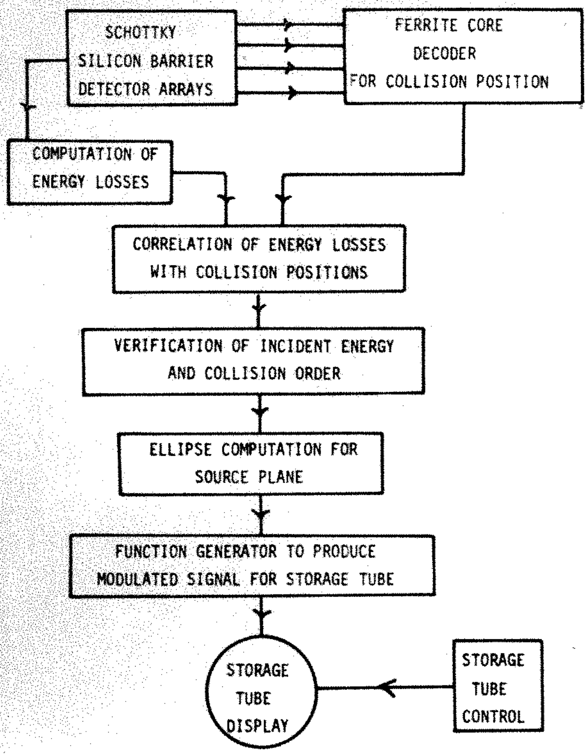

Fig. 3 Schematic representation of the proposed system.

The detector must be capable of measuring the coordinates and intensity of an interaction, and liquid or solid scintillators, proportional chambers and semiconductor detectors would be suitable. Energy resolution, response time and transmission properties suggest, however, that the latter offers the best solution and a prototype is under construction, based on a lattice of Schottky barrier orthogonal arrays of 0.5 mm cubic elements. Because the device uses Compton scattering, it is not necessary to use high atomic number detectors working at very low temperatures.

It is normal in image processing to define the optical accuracy in terms of a point spread function; a complete image is the result of convolution with source distribution. In collimator systems there is a one–to–one correspondence between source points and a mean point on the image plane. The point-spread function gives the statistical spread about this mean. In the proposed system a source point generates a deterministic ellipse on the image plane, in the absence of measurement errors. It is possible to determine the statistical spread, resulting from measurement errors, about the ellipse. This distribution is not, however, directly useful in an assessment of image formation and, in practice, each image point is obtained by an accumulation of ellipses. It is therefore more appropriate to define a point spread function for the sum of ellipses about their common point of intersection. Figure 4 shows the theoretical point spread function assuming the source to be 10 cm from the detector. This is a more pessimistic estimate than the so-called intrinsic point spread function.

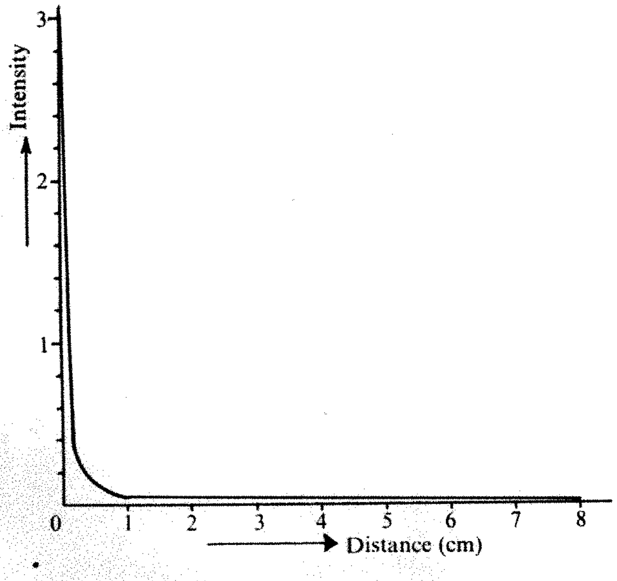

Fig. 4 Point spread function of the Compton camera.

The point spread function used here assumes statistical stability. That is, the addition of further ellipses does not improve the function. Although this results in a sharp distribution, it is fair because comparisons with other systems allow consideration of the number of counts necessary to form an image. In more conventional measurement terms this is concerned with the relationship between signal : noise ratio and the integration time. To achieve this evaluation in a meaningful way a uniform source sheet with a small hole at its centre is considered (Fig. 5). The image contours will not have a discontinuity corresponding to the hole edge but will vary gradually to a minimal value at the centre of the hole. Detection of the hole therefore depends on the fractional image density difference between the outside and the interior of the hole (Fig. 5).

The other significant difference between the proposed system and the collimator system is that data processing is an integral part of the optical system. In the Compton system it is possible to effectively improve the optical properties of the detector by

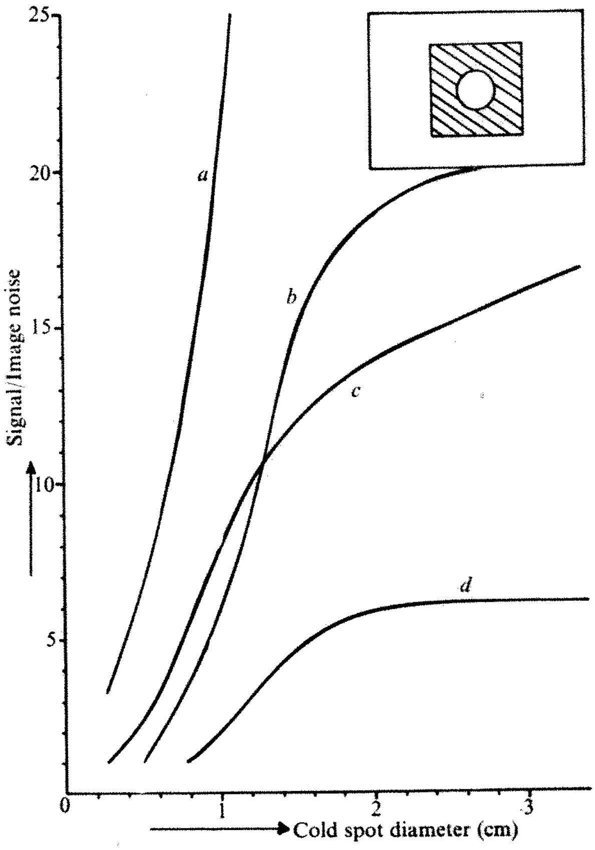

Fig. 5 Signal to image noise ratio against the cold spot diameter. *a*, Compton system, 10^6 ellipses (10 s); *b*, collimated system, 10^6 counts (1,000 s); *c*, Compton system, 10^5 ellipses (1 s); *d*, collimated system, 10^5 counts (100 s). Imaging times refer to a 400 Ci source; average source activity is one third of peak activity; there was no image processing. Inset: radioactive source sheet with a hole at its centre.

operating on the incoming data during the formation of the image. In the collimator systems, data processing is normally only applied to the completed image.

If it were necessary to draw each individual ellipse the display problem would be immense. Using statistical estimation, however, it seems possible to produce best estimates of the intersection point without having to display each ellipse. The extra computation involved is extremely small.

A number of practical problems arise which have led to further studies. These include the determination of the sequence of interactions, the removal of spurious data by time coincidence methods, amplification of a large number of low intensity pulses, and detector fabrication. Research carried out so far indicates that none of these problems have any serious implication to the basic concept.

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Carbon isotope fractionations in natural gases

THE origin of gaseous hydrocarbons has been considerably illuminated by isotope analyses of natural gases. We shall try here to establish a model, explaining the isotopic fractionations of methane as a function of the thermocatalytic development of the corresponding source material. Because the origin of gaseous hydrocarbons is closely related to the formation of crude oil on one hand and the thermocatalytic state of the organic source material on the other, isotopic investigations can be of great interest in exploration for hydrocarbons.

The results of isotope analyses of natural gases from Japan[1], Taiwan[2], Italy[3] and the DDR[4] are compared with isotope measurements of gases from the Federal Republic of Germany[5]

The German gases were taken from different geological formations and wells of the north-western German oil and gas province. The methane (C_1) of the sampled gases was separated from the remaining gas components by liquid oxygen separation[5], combusted to carbon dioxide and its carbon isotope composition determined in a mass spectrometer (Atlas-Varian M 86). The isotope ratios are given in the usual δ notation:

$$\delta^{13}C_1 = [(^{13}C/^{12}C)_{\text{sample}} - (^{13}C/^{12}C)_{\text{standard}}]/(^{13}C/^{12}C)_{\text{standard}} \times 1000(‰)$$

The reference gas (standard) is the PDB-standard, which is representative of the isotopic composition of a marine carbonate. The reproducibility of the δ values is ± 0.3‰. The $\delta^{13}C_1$ values of dry natural gases are shown in Fig. 1 and plotted against the ratio of methane (C_1) over all saturated gaseous hydrocarbons (ΣC_n). Gases associated with oil or with high carbon dioxide content are omitted because of possible isotope exchange fractionations.

Biogenic alteration of the organic matter is predominant during the first few feet of sedimentary burial. Bacterial action on the organic material results in methane enriched in the light ^{12}C isotope[6]. Further sedimentation brings the organic material to a greater depth and thus into higher temperature zones. Chemical processes such as hydrolysis, cracking and hydrogen disproportion become more and more important. Reaction rates double for every 10° C rise in temperature. Isotopic fractionation occurs as a result of the different binding energies of the various isotopic molecules: ^{12}C–^{12}C bonds are the first bonds to be destroyed by thermal stress[7]. The methane emerging from the first stages of thermocatalytic alteration of source material is therefore rich in the light isotopes. Consequently the 'young' gases from Japan and Southern Italy (Fig. 1) are characterised by large amounts of methane ($C_1/\Sigma C_n > 0.99$) with low concentrations of ^{13}C ($\delta^{13}C_1 < -60‰$). With deeper burial, as the thermal stress on carbon complexes increases, more ^{13}C–^{12}C bonds are broken and the gas becomes isotopically heavier. Increasing thermocatalysis also causes the ratio of methane to all saturated gaseous hydrocarbons ($C_1/\Sigma C_n$) to change in favour of the higher hydrocarbons[8]. The extent of thermocatalytic change is dependent on temperature, that is, on the depth of the source bed formation, the time of maximum temperature influence, and the time when the reservoir rock became a closed system. Therefore, the degree of thermocatalytic alteration of organic material is sometimes linked to the geological age of the source bed formation and, subsequently, to the age or depth of the reservoir bed of the natural gases. This trend is shown by the data of the Quaternary Japanese, the Tertiary Southern Italian and the Permian or Triassic natural gases from the Thuringian Basin (Fig. 1), in which the source bed material is of predominantly marine origin. A turning point in the development of marine organic material is represented by the data on Permian natural gases from the Thuringian Basin, ($C_1/\Sigma C_n \approx 0.8$; $\delta^{13}C_1 \approx -42‰$). As the organic source material is further altered, the hydrocarbons released

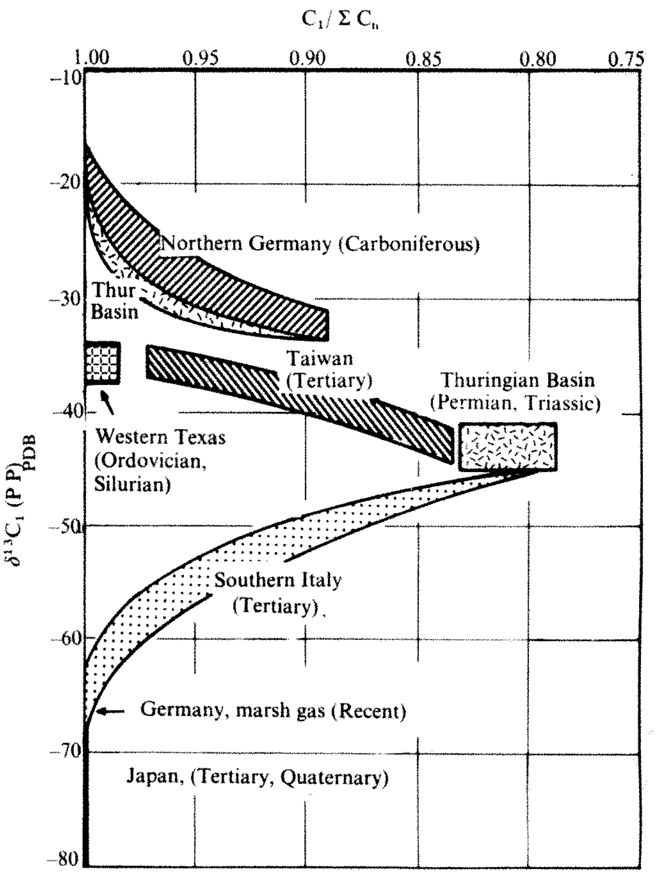

Fig. 1 Isotopic variations in methane in natural gases (refs 1–5, 9 and W. Stahl and B. D. Carey, in preparation).

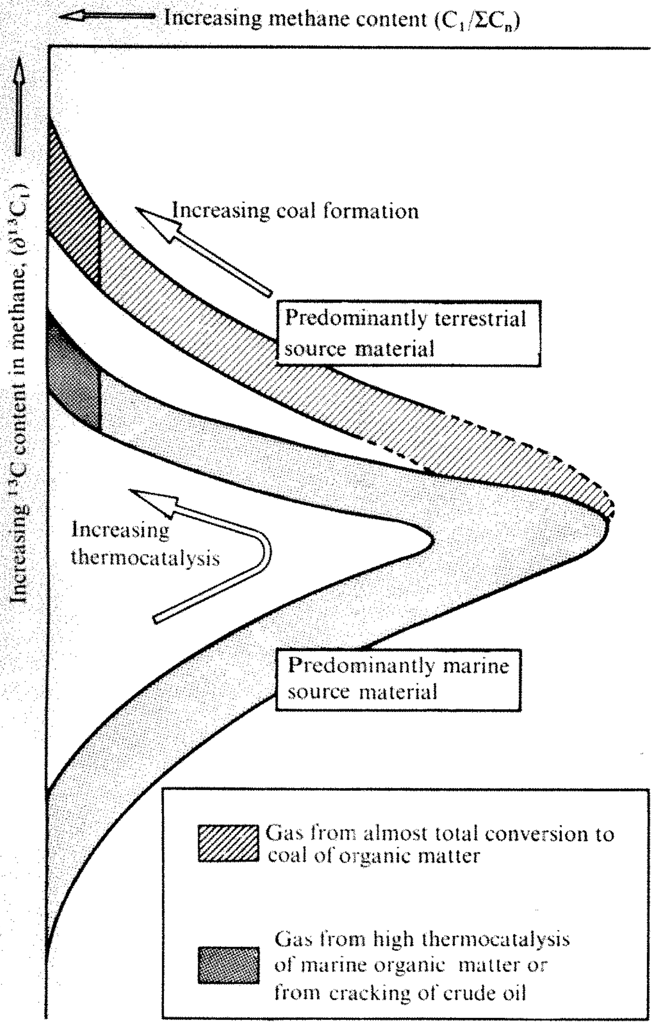
Increasing methane content ($C_1/\Sigma C_n$)
Increasing ^{13}C content in methane, ($\delta^{13}C_1$)
Increasing coal formation
Predominantly terrestrial source material
Increasing thermocatalysis
Predominantly marine source material
Gas from almost total conversion to coal of organic matter
Gas from high thermocatalysis of marine organic matter or from cracking of crude oil

Fig. 2 Fractionation model.

are increasingly cracked, and the methane content of the gas and the $^{13}C/^{12}C$ ratio of the methane increases. This stage is represented by the Taiwan data. The thermocatalytic development of this source material has been accelerated by magmatic events during the Tertiary. In the final stage, all higher hydrocarbons are converted into methane by cracking, enriched in the heavy isotope. This final stage of thermocatalysis is shown by the analytical data of deep dry Ordovician and Silurian gases from West Texas (W. Stahl and B. D. Carey, in preparation).

On the other hand, the methane content of the gases produced during the formation of coal, for example the Carboniferous, Permian and Triassic natural gases of the Federal Republic of Germany, is also considerably enriched in ^{13}C. These gases, formed when predominantly terrestrial organic material is converted to coal, show properties comparable with gases from the cracking stage of marine source material: the $\delta^{13}C_1$ values change with increasing thermal stress in favour of the ^{13}C isotope, methane content increases, and the amount of higher hydrocarbons decreases with increasing degrees of conversion to coal.

The observations discussed here are summarised in Fig. 2, where an economically important application of the model is demonstrated, the distinction of pure methane gases of different origin. The origin of methane gases characterised by low δ values, that is enriched in ^{12}C, can be traced back to young organic material, which was only affected by relatively low temperatures. High ^{13}C concentrations in methane show that the gas originated during coal formation processes or during the thermal destruction of marine organic matter or liquid hydrocarbons under relatively high temperatures. In this case, the probability of finding crude oil will be extremely small.

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Evidence on the locomotor pattern of *Homo* from early Pleistocene of Kenya

Here I present the results of the application of two multivariate statistical techniques, a linear function and a distance statistic, in the analysis of a hominid talus bone. They provide additional objective evidence that this talus, found at East Rudolf, North Kenya, in 1971, should be included in the genus *Homo*. They also indicate that the morphology of the bone is compatible with early *Homo* having a lower limb functioning like that of modern man.

The specimen, KNM-ER813 (ref. 1), is a well preserved right talus; details of its morphology have been described and illustrated elsewhere[2]. It was recovered from beds between tuffaceous horizons that have been correlated with tuffs dated at 2.61±0.26 and 1.57±0.00 million yr (Myr) by argon isotope techniques[3].

Comparative data which have been used as a reference framework are listed in Table 2 and details of the groups have been given in previous reports[4,5]. Three angular and five linear measurements were made on 318 adult tali. These measurements relate to the size and disposition of the articular surfaces, and were selected both for their reproducibility and for their biomechanical significance in locomotion. The five linear measurements were made up into four indices to reduce the effects of overall size disparity in bones that are of the same shape. The values of the variables for the fossil are given in Table 1 (see ref. 4 for further details of measurements).

Canonical analysis of these data, using the BMD 07M program[6], resulted in five significant canonical variates. Variate I, containing 76% of the total variability, isolated the modern human tali; variate II separated the knuckle-walking category; the two subsequent variates separated the arboreal forms, from the rest (III), and from each other (IV); variate V was nondiscriminatory.

The canonical coefficients of these first four variates have been applied to the fossil data, a procedure that is quite proper[7]. In all the variates, the fossil is aligned with the modern human tali and in the first canonical axis (which by definition effects the greatest measure of separation) the fossil is especially close to man (Fig. 1).

Table 1 Data for the fossil talus

Variables	Horizontal angle	Dip angle	Torsion angle	Neck length index	Head projection index	Trochlear shape index	Fibular facet projection index
KNM-ER813	14	25.5	48	130	40	91	222

The Mahalanobis D^2 distances of the fossil from the comparative groups are given in Table 2. The distances between the fossil and all three groups of modern human tali are small, so small in the cases of the Bushmen and the Modern groups that the distances are not statistically significant. These D^2 results indicate that the behaviour of the fossil in the canonical variates, in particular in variate I, are a true reflection of its affinities (Oxnard has recently pointed out that this may not be the case where large and significant D^2 distances are involved[8]).

Although the multivariate analysis results are expressed in fewer dimensions than there are measurements, they confirm the close morphological similarity that exists between the fossil and modern human tali. Their relevance for its classification are that they strengthen preliminary assessments which placed this fossil talus in the genus *Homo*[1,2]. There is evidence from other fossils that the *Homo* lineage is present at East Rudolf in deposits both older and younger than those in which this fossil was found[1,9]. It cannot be certain to which grade or chronospecies of *Homo* this talus belongs for no talus has been found associated with fossils characteristic of the 'erectus' grade of the *Homo* lineage. Two femur shafts, however, KNM-ER737 and 803, that are broadly contemporaneous with the talus, show features characteristic of the *Homo erectus* femurs from Choukoutient and Olduvai Gorge[10].

Considering the locomotor implications of these results, although the modern human locomotor pattern seems to be associated with a particular talar morphology, one would be guilty of the *post-hoc* fallacy in assuming that a similar talus was necessarily part of the same sort of hind limb functional complex. Nevertheless, with that caveat, the functional implications of the canonical analysis results, combined with the close morphological affinity of the fossil talus with the modern human bones, make it probable that the locomotor pattern of this early hominid was like that of modern man. More comparative and functional data from other bones in the lower limb are needed to confirm this hypothesis.

One of the objects of accumulating comparative material has been that the groups could be used as a framework to compare fossil bones. Similar fossils would be associated on the canonical variates and their D^2 distances from the comparative groups should be similar. It is of particular interest, therefore, that this East Rudolf talus and a talus of a similar time period, OH8 from Olduvai Gorge, Tanzania, behave differently in the analysis[4]. The suggested implications of these differences for the classification and phylogenetic interpretation of the Olduvai specimen will be presented elsewhere.

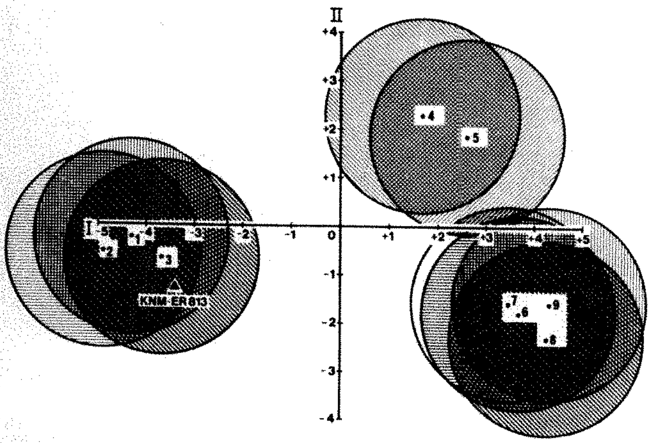

Fig. 1 Plot of canonical variates I and II. Circles are drawn with a radius of 2 s.d. The group numbers are identified in Table 2.

Table 2 D^2 distances and probability levels between special case and groups

No.	Group	
1	*Homo sapiens* (Modern)	14.6 5.0%
2	*Homo sapiens* (Anglo-Saxon)	21.8 $<0.005\%$
3	*Homo sapiens* (Bushmen)	11.5 10.0%
4	*Gorilla*	56.4 $<0.005\%$
5	*Pan*	57.6 $<0.005\%$
6	*Papio*	76.45 $<0.005\%$
7	*Cercopithecus*	67.6 $<0.005\%$
8	*Colobus*	63.6 $<0.005\%$
9	*Pongo*	88.1 $<0.005\%$

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Coal smoke and mortality of the elderly

MORTALITY from falls in old people is so closely related to mortality from fractures of long bones, particularly of the proximal femur, that it seems to be related to bone fragility[1–3]. Although the mortality of women aged 75 yr and over is more than 50% higher than that of old men, there has been a close relationship between male and female annual mortalities in both Britain and the United States. This suggests that some common contemporary factors affected the mortality, and possibly the bone fragility, of each sex, even though their total experiences of mortality were substantially different. The evidence presented here suggests that coal smoke is one such factor.

A striking feature of mortality from falls of the elderly in Britain has been the recession in mortality over the period of wartime and post-war austerity from 1940 to 1950, subsequently rising to be followed later by a steady fall since 1958–59. This pattern, which appears in the mortalities of both men and women aged 75 yr and more[1,2], resembles that shown by annual estimates of atmospheric pollution by domestic coal smoke. This is illustrated in Fig. 1.

The estimates of annual emission of smoke from house coal shown in Fig. 1 were obtained, together with estimates of railway smoke from steam locomotives and industrial smoke, from estimates given by the Warren Spring Laboratory[4] for the years 1952–65 based on data of coal consumption. For years before and after that period the factors used to make these estimates (weight of smoke = 3.5% of domestic coal, 2% railway coal, 0.5–1.2% industrial coal) were applied to data of annual coal consumption compiled for those years[5]. The relationships of mortality to estimated annual emission of coal smoke are shown in Table 1. Mortalities are related to domestic smoke pollution for both men and women but only for bronchitis in women to industrial smoke, the highest correlations being between domestic smoke and falls, both in men ($r = 0.73$) and in women ($r = 0.82$).

The immediate cause of death of an elderly person immobilised by a fractured femur is usually a lung infection. The mortality of middle-aged people up to the age of 75 yr in County Boroughs has been related, *inter alia*, to estimates of domestic smoke pollution in the different boroughs, a principal cause of the relationship being mortality from bronchitis[6,7].

The mortality from bronchitis of women aged over 75 yr is shown in Table 1 to be highly correlated with mortality from falls ($r = 0.70$) and with domestic smoke ($r = 0.78$), bronchitis in men has lower correlation coefficients both with falls ($r = 0.51$) and smoke ($r = 0.37$). Mortality of men from bronchitis is higher than that of women, and additional factors, particularly tobacco, affect their mortality from bronchitis. The marked recession in mortality from falls in 1940–50 followed by post-war peaks in 1956 and 1958–59 did not occur in mortality from bronchitis, but the annual fluctuations of mortality from bronchitis were reduplicated at a lower amplitude by the mortality from falls. There was an increased risk of death following a fall in bad years for bronchitis.

Table 1 Correlations and multiple regressions of death rates from falls with death rates from bronchitis and domestic smoke pollution

Coefficients of linear correlation (r)		Bronchitis mortality i	Domestic smoke ii	Industrial smoke iii
		r	r	r
Bronchitis mortality	male		0.37*	0.17‡
	female		0.78†	0.63†
Falls mortality	male	0.51*	0.73†	0.12‡
	female	0.70†	0.82†	0.17‡
Multiple regression				
Partial regression coefficients (b)		b_{i}	b_{ii}	
Falls mortality	male	0.046*	0.479†	
	female	0.024‡	0.721†	

For the multiple regression the multiple correlation coefficient, R was 0.78† for men and 0.83† for women.

Industrial smoke includes railway smoke.

* $P<0.05$

† $P<0.001$

‡ Not significant

Analysis by multiple regression with mortality from falls as the dependent variable and two independent variables—domestic smoke pollution and mortality due to bronchitis—gives highly significant regression coefficients for smoke pollution both in men and women ($P<0.001$), the regression coefficient for bronchitis for women is not statistically significant ($0.25<P<0.4$) but is probably significant for men ($P = 0.02$) at a much lower significance than for smoke pollution. Mortality from falls (and by inference from fractures of the long bones) seems, therefore, to be related to domestic smoke pollution independently of the relationship to mortality from bronchitis.

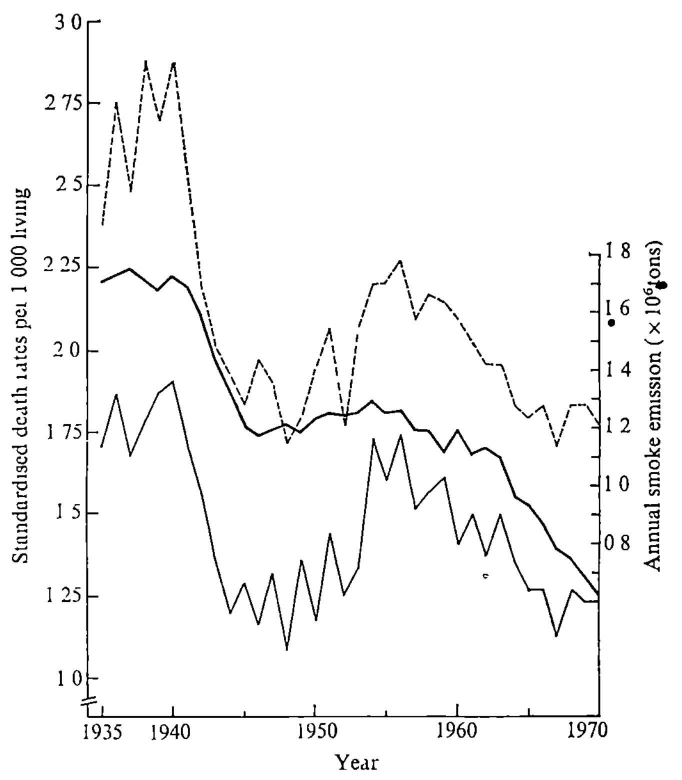

Fig. 1 Death rates at age 75 yr and over for accidental falls (E880-7 eighth revision International Classification of Diseases) compared with estimated annual emission of domestic coal smoke. Death rates are directly standardised for age and sex on the total male and female 1961 population at age. —— Male death rates, – – – female death rates, —— smoke emission.

The steep rise in femoral fractures with advancing age in Britain may be attributable to an absolute or relative deficiency of vitamin D (refs 8 and 9). In Detroit (latitude 42°N) serum antirachitic activity, as well as being related to season and sunshine, was significantly less in symptomatic osteoporotic subjects than in the skeletally normal[10]. There is increasing evidence of the importance of endogenous synthesis of vitamin D by ultraviolet irradiation of 7-dehydrocholesterol in the skin[11], even in the higher latitude of London (52°N) where there are seasonal changes in plasma levels of 25-hydroxycholecalciferol[12]. When smoke pollution was at its height in 1937–39, it was estimated that in Leicester (latitude 53°N) at least 30% more ultraviolet daylight would have reached the centre of the city in winter if all the smoke in the atmosphere had been eliminated, and occasionally the loss was more than half[13]. The effect of smoke on mortality from falls and fractures may therefore have been due to reduced endogenous synthesis by ultraviolet light.

These relationships between mortality and environmental change have implications beyond the restricted area of geriatric mortality. Because they are a dying generation at the end of the human life span, factors affecting the inevitably high mortality and causes of death of people over 75 yr of age are fewer and simpler than the much more complex influences affecting the lower mortalities of middle age. Though they will succumb to illnesses and infections which would be trivial and easily treated in a younger person, they will have survived or no longer be subjected to many of the stresses and influences competing for life or death affecting mortality and morbidity in middle age They may therefore show relationships of mortality to single factors in environmental change which are masked by complex multifactorial influences at younger ages The simpler relationship of mortality from bronchitis in women to both domestic and industrial smoke is particularly notable

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Genetics of cannibalism in a viviparous fish and its relationship to population density

THE success of viviparous species is a result of their capacity to produce small numbers of advanced offspring with high survivorship potential Cannibalism, which would appear to be antithetical to this adaptation, is widespread among viviparous fishes[1–3] I have examined the genetics of cannibalism and the significance of its density-dependent mechanism in teleosts of the genus *Poeciliopsis* A five-membered unisexual–bisexual complex has evolved in this genus through hybridisation of a cannibalistic species, *Poeciliopsis monacha*, with the noncannibalistic species, *P lucida* Hybridisation of these two species formed *P monacha-lucida*[4], a diploid unisexual, backcrosses of this diploid form with each of its progenitors resulted in the evolution of two triploid unisexuals *P monacha*-2 *lucida* and *P* 2 *monacha-lucida*[5] All these rely on males of the bisexual species for fertilisation

The unisexual–bisexual complex represents an array of morphological and biochemical characters[5,6] that demonstrate the genetic intermediacy of unisexuals according to genome dosage of parental forms I have investigated whether the degree of cannibalism also follows genome dosage and whether adult and/or young densities influence the expression of this trait

Live and preserved stocks of *Poeciliopsis* were obtained from natural populations in the Rio del Fuerte of Sonora and Sinaloa, Mexico Mature virgin females were maintained in 75 7-l aquaria with sparse amounts of *Najas* and *Vallisneria*, many snails, and fed twice daily with commercial dry food supplemented with live *Daphnia* Females were established at densities of five, ten, fifteen and twenty, plus a control with no females

Young fish obtained from stock females were screened for developmental abnormalities on the day of birth, and then introduced at densities of five, ten, fifteen and twenty into the experimental aquaria containing the mature females Most young were of the same brood, however, samples of fifteen and twenty young were formed occasionally by combining broods of different females Young remained with the predators for 24 h, after which survivors were removed and counted Preliminary experimentation demonstrated that young would not be cannibalised after 24 h Mortality in fifty controls each of 24 h duration was zero

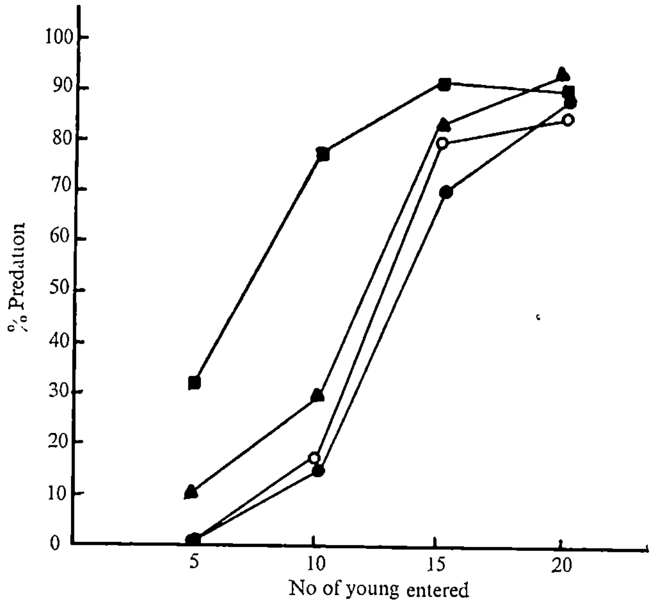

Fig. 1 Relationship of predator and prey densities to predation rates Females of *Poeciliopsis monacha-lucida*, *P* 2 *monacha-lucida* and *P monacha* served as predators of newborn young of all five species of *Poeciliopsis* These females were used because of large sample sizes and their consistent cannibalistic behaviour Densities of females were ●, five, ○, ten, ■, fifteen, ▲, twenty

Among the five unisexual–bisexual *Poeciliopsis, P lucida* proved to be noncannibalistic whereas *P monacha* consumed young in nearly all trials (Table 1) *P monacha-lucida*, the genetically intermediate hybrid, ate young in about three out of four trials The two triploids cannibalised at rates similar to that of the bisexual species to which they are genetically more similar *P monacha*-2 *lucida* ate young nearly as infrequently as *P lucida*, *P* 2 *monacha-lucida* nearly as avidly as *P monacha* The degree of cannibalism, like morphological expression, thus comprises an array directly related to genome dosage This is the first time the predatory phenomenon has been demonstrated to be heritable Stomach analyses of wild females confirm that certain species of *Poeciliopsis* eat young and that cannibalism is not a laboratory artefact

Cannibalism, believed to be polygenically controlled, is a complex behavioural phenomenon related to feeding habits The practice of cannibalism by *P monacha* and not by *P lucida* is probably related to their different morphology and ecology Dentitions, for example, differ *P monacha* has many tricuspid inner teeth whereas *P lucida* has few unicuspid inner teeth[5] *P monacha* is found in small headwater pools, *P lucida* in large and stable tributaries

Table 1 Frequencies for which any young were cannibalised by unisexual–bisexual *Poeciliopsis* in 136 trials

Species	Total no of trials	No of trials in which young were cannibalised	*F*
P lucida	19	0	0 0
P monacha-2 *lucida*	17	2	0 12
P monacha-lucida	54	40	0 74
P 2 *monacha-lucida*	25	22	0 88
P monacha	21	20	0 95

Table 2 Probability model comparing total survivorship potential of young produced by fishes with superfoetation and fishes without superfoetation in a catastrophic environment

Intervals of 10 d (total 100 d)	1	2	3	4	5	6	7	8	9	10
Young production with superfoetation	10	10	10	10	10	10	10	10	10	10
Young production without superfoetation	20	0	20	0	20	0	20	0	20	0

$$P_x = (n!/(x!(n-x)!))\,(p^{n-x}q^x)$$

$P_x = (\quad)$	× (No of young)	= (total young surviving)	$P_x = (\quad)$	× (No of young)	= (total young surviving)
$P_0 =$ 0 349	× 100	= 34 9	$P_0 =$ 0 59	× 100	= 59 0
$P_1 =$ 0 387	× 90	= 34 83	$P_1 =$ 0 328	× 80	= 26 24
$P_2 =$ 0 194	× 80	= 15 52	$P_2 =$ 0 073	× 60	= 4 38
$P_3 =$ 0 057	× 70	= 3 99	$P_3 =$ 0 008	× 40	= 0 32
$P_4 =$ 0 011	× 60	= 0 66	$P_4 =$ 0	× 20	= 0
$P_5 =$ 0 001	× 50	= 0 05	$P_5 =$ 0	× 0	= 0
P_6 10 = 0	× No of young	= 0			

$$\sum_{x=0}^{10} (P_x \times \text{No of young}) = \text{total No young surviving in 100 d} = 89\ 95 \qquad \sum_{x=0}^{5} (P_x \times \text{No of young}) = \text{total No young surviving in 100 d} = 89\ 94$$

The probability of a catastrophe occurring in any one day is 0 1 n is the total number of broods, x is the number of broods lost, p is the probability of a brood surviving (0 9) q is the probability of a brood lost (0 1), P_x is the probability of x number of broods being lost

Previous experimentation with *Poecilia reticulata* on cannibalism and fecundity under crowded conditions, supported the hypothesis for a water-borne substance that acted to reduce fecundity or increase the cannibalistic response[2] These conclusions do not seem to be applicable to *Poeciliopsis* populations Also, because cannibalism had previously been proposed as a density-dependent population regulatory mechanism in *Poeciliopsis*[7], I analysed my results with respect to variable numbers of predator and prey (Fig 1)

Intensity of predation is a function of the number of females present the more females are present, the more young will be consumed But more critically, intensity of predation is a function of the number of young introduced At densities of five or ten young, predation clustered at 0–25% regardless of female density With fifteen to twenty young, cannibalism increased to 70–90% For 128 trials, a two-way analysis of variance was used (F statistic highly significant ($P \leqslant 0\ 001$) for young density and ($P<0\ 001$) for female density)

The hypothetical value for the 'critical density' of between ten and fifteen young is consistent with laboratory observations of females preying on young When more than ten young were introduced to aquaria containing adult females, the young tended to school or aggregate near the surface, a characteristic of newborn *Poeciliopsis* Aggregates of young stimulate an attack response first by one female followed by mobbing behaviour from the others After the initial attack, young quickly disperse in the vegetation or remain immobile, in the absence of schools, the searching response by the females gradually subsides over the next few hours

Aggregates of ten young or fewer do not stimulate a predatory reaction, however, they are eaten at random when encountered by a female, thus explaining the relationship of the number of females to the rate of cannibalism Cannibalism is less related to hunger than to predator–prey density phenomena Females starved for 2–4 d ate no more young than did well-fed females Furthermore, females encountering large densities of young remain unsatiated until the brood is eradicated Discrimination of young is not involved In a mixed environment, the probability of an individual eating its own young is low since cannibalism is suppressed until parturition is completed

The adaptive value of cannibalism is unclear Experimentation indicates advantage for lower densities of young Newborn young emerging in smaller groups should have higher survivorship values than young born in larger groups

All species of *Poeciliopsis* exhibit superfoetation, the simultaneous development within the ovary of more than one embryonic stage *P lucida, P monacha* and associated unisexuals produce half as many young twice as often as females of the same size of *Poecilia reticulata* which do not have superfoetation The former have mean brood intervals of 11 6 and 10 3 d respectively, whereas the latter has 21 1 d, *P monacha* and *P lucida* females of 30 to 35 mm total length have mean brood sizes of 11 8 and 11 0 young respectively compared with 30–35 mm *P reticulata* females with broods of 24 2 young[8] (unpublished work of myself and R J Schultz) The birth of small broods at frequent intervals may be an adaptation to a critical density for cannibalistic predation

Superfoetation is believed to have evolved independently in response to various selection pressures[9–10] Several theories on the adaptive value of superfoetation appear reasonable, but they break down under close scrutiny Superfoetation may allow females to utilise space devoted to reproduction more efficiently by partitioning the young into different developmental states, thereby increasing total fecundity, however, fishes without superfoetation have attained brood sizes of 180 and 315 young[11] far exceeding twice the maximum brood size of 80 young of *P monacha* females of comparable sizes (unpublished work of myself and R J Schultz)

Superfoetation may also be beneficial to fishes inhabiting unstable environments such as those of *Poeciliopsis* which are characterised by floods, droughts and extreme temperatures, all with the potential of eradicating an entire brood of young By scattering broods of smaller numbers, young of fishes with superfoetation may exhibit higher survivorship potential But, a simple probability model contrasts the reproductive potential of fishes with superfoetation with that of fishes without superfoetation in a catastrophic environment where catastrophies are random and unpredictable (Table 2) In this design, both types of fish have equal probability of producing the same numbers of young in a set interval superfoetation yields no advantage

The adaptive value of superfoetation is unclear In spite of the function of cannibalism in the regulation of population size, the adaptiveness is also ambiguous However, superfoetation and cannibalism are correlated in the producton and survivorship of small broods in *Poeciliopsis* Cannibalism is probably uninvolved as a selective force in the evolution or maintenance of superfoetation, its persistence in viviparous fishes without superfoetation supports this But with the presence of the numerical predator–prey mechanism, cannibalism appears to be tolerated in the evolution of natural populations when superfoetation is present as a reproductive strategy

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Depth perception in disparity gratings

OBSERVATION of sinusoidal spatial modulation of luminance has proved a valuable tool in the study of visual processing of luminance distributions on the retina Information on the limits of processing capability has been gained in such diverse fields as the optics of the eye[1], neurophysiology of the retina[2], movement[3,4] and orientation[5 6] sensitivity and cortical processing of spatial frequency[7 8] In the field of stereoscopic vision, Blakemore[9] and Fiorentini and Maffei[10] have found that when luminance gratings of slightly different spatial frequencies were presented to each eye, an apparently tilted grating was perceived even if one monocular grating was moving quickly relative to the other Furthermore, tilt is perceived from gratings of the same spatial frequency but differing in contrast by 50% or more These phenomena are not easy to explain on the basis of disparities at corresponding retinal points, but seem to require more global processing of the whole image

Julesz[11] has reached a similar conclusion on the basis of experiments with random-dot stereograms, which have the unique advantage of eliminating all correlated monocular cues to the stereoscopic figure (although the random dot texture acts as a monocular textural cue that the figure has a constant depth and works against the disparity information to some extent) Stereopsis can be obtained under several conditions which make it difficult to conceive of a local disparity process that could produce depth perception For example, alternation of two stereograms at 20 Hz does not destroy perception of a stereoscopic figure[12] Furthermore, in ambiguous stereograms the depth perceived depends on the global organisation of nearby dots[13]

It would be of value to combine the advantages of random-dot stereograms and of sinusoidal modulation techniques in the study of spatial limitations of cyclopean depth perception I have previously studied the effects of sinusoidal modulation of binocular disparity along a vertical axis on stereoscopic perception using line stereograms[14] The main stereoscopic processing limitations noted were a sharp high frequency limitation in sensitivity at about three cycles per degree (c p d) and a disparity scaling effect, in which the maximum disparity that could evoke a depth percept was directly proportional to the retinal angle subtended by each cycle of the vertical modulation Thus, the disparity limit is scaled in proportion to

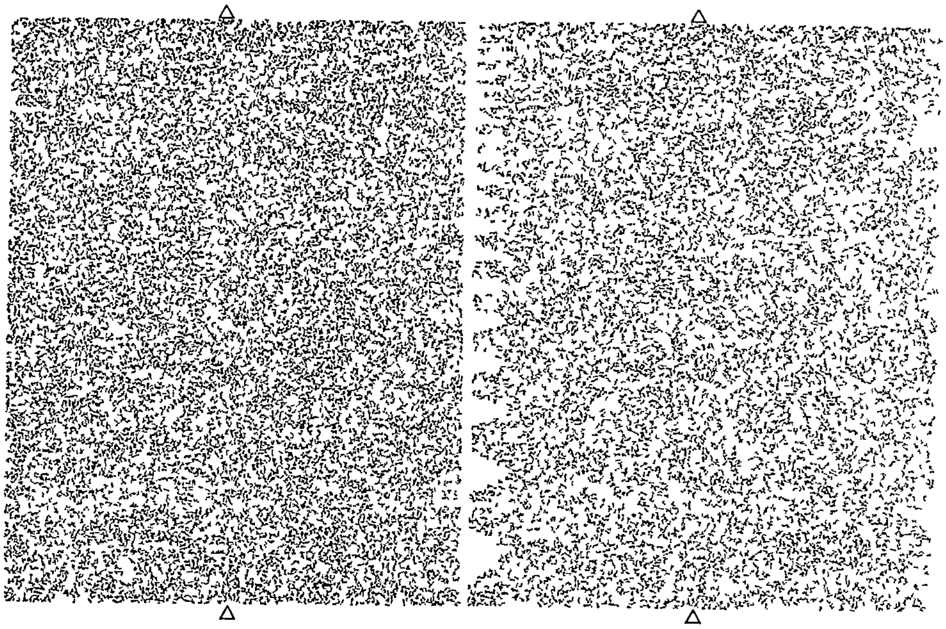

Fig 1 Demonstration stereograting This figure should be viewed by means of a simple stereoviewer requiring two white cards and a pair of spectacles With the figure at arm's length hold the cards at about 10 inches from the eyes Closing each eye in turn, align the cards so that each eye sees only one dot matrix It should now be possible with the aid of the fixation marks to register the two matrix images as a single square If this square remains blurred it may help to wear a pair of weakly hyperopic spectacles (in addition to any spectacles worn normally) Continued observation should reveal shallow horizontal ripples or cones increasing in spatial frequency upwards and becoming shallower towards the right Generation of random-dot disparity gratings was achieved by a Fortran program controlling the position of randomly selected points in a 10 × 8 inch matrix on a Calcomp plotter In measuring stereoscopic thresholds the problem that dot resolution is about 1 arc min visual angle, whereas disparity resolution requires down to 10 arc s, was solved by modifying the concept of a completely random matrix so that dot position was specified to one tenth of a dot width This gave minimum possible disparity shifts of about 13 arc s at the viewing distance of 200 cm Although horizontal changes in disparity theoretically introduce slight monocular cues to the disparity information, such cues are negligible in practice, since increasing the disparity range to ten times the largest used in this study did not introduce detectable monocular variation in dot density

size of the modulation cycles The experiments reported here show that the line stereogram results are replicated using random-dot stereogratings as stimuli and are probably general properties of the stereoscopic system

Generation of random-dot disparity gratings with a large range of disparity variation required a slight modification of Julesz's concept[11] (see Fig 1) A second technique enabled threshold sensitivity to be measured over a great range of amplitudes and frequencies of disparity modulation without generating a vast number of individual stereograms By analogy with the demonstration stimulus for luminance grating sensitivity[15] it is possible to produce continuous variations of both amplitude of disparity and spatial frequency as a frequency-swept, amplitude-decayed stereograting (Fig 1) The boundary between perception of the flat random-dot texture and the rippled surface of the stereograting is determined by the spatial frequency limits of disparity processing in the visual cortex

For demonstration, the monocular cues of the disparity ripple at the edges of the right hand matrix is present, but these were masked to straight edges during quantitative experiments The edge ripple in relation to the straight edge of the left-hand matrix indicates the precise disparity present down each edge of the figure Readers may verify that no changes in texture density correlated with the disparity variations are evident in Fig 1

To ensure that maximal stereoscopic information was available, free eye movements and long and active viewing times were encouraged The stereogratings were viewed through a Smith orthorater stereoscope Each subject was fully optically corrected, since individual dots were close to the limit of visual resolution The stimuli were Xerox copies of the Calcomp plot which had a luminance of 3 cd m^{-2} and were viewed at a distance of 200 cm Subjects were instructed to concentrate on each bar indicated in turn, and decide where in the matrix the bar disappeared into a flat surface They then directed the experimenter in marking the point on the matrix with a red mark invisible to the subject A complete set of spatial frequencies was measured twice

The results of the measurements of spatial frequency limitations of stereosensitivity for two subjects (Fig 2) are in general agreement with line stereosensitivity limits[14] The maximum frequency for which the stereograting can be resolved (Fig 2*a*) is about 4 c p d (compared with 3 c p d in line stereograms[14]), and is only slightly affected by a tenfold reduction in dot density (Note that at this frequency there are 7 dot widths or 70 dot positions per cycle) The slight discrepancy between the uppermost points in Fig 2*a* and the lowermost in Fig 2*b* is probably due to measurement error and may also be influenced by edge effects at the edge of the grating

Similar results may be obtained for previously published stereograms containing both vertical and horizontal gratings[16] The grating becomes invisible as viewing distance is increased so that the grating subtends about 4 c p d This is not due to lack of acuity for the monocular textural information, since in stereograms of the same texture at the same viewing distance a large stereoscopic figure such as a square is readily visible

High spatial frequency sensitivity may also be studied by removing high or low spatial frequency information from one matrix of a stereogram[16] Once again stereopsis is difficult (impossible for all five observers I tested) when there are no low spatial frequency cues to the stereoscopic figure, whereas lack of high spatial frequency information hardly affects stereoscopic perception

As with all measures of stereolimits there is a lower and an upper disparity for which perception of depth falls to threshold The former will be called stereoacuity and the latter the upper depth limit Stereoacuity falls in the region of the resolution of the Calcomp plotter, so it could not

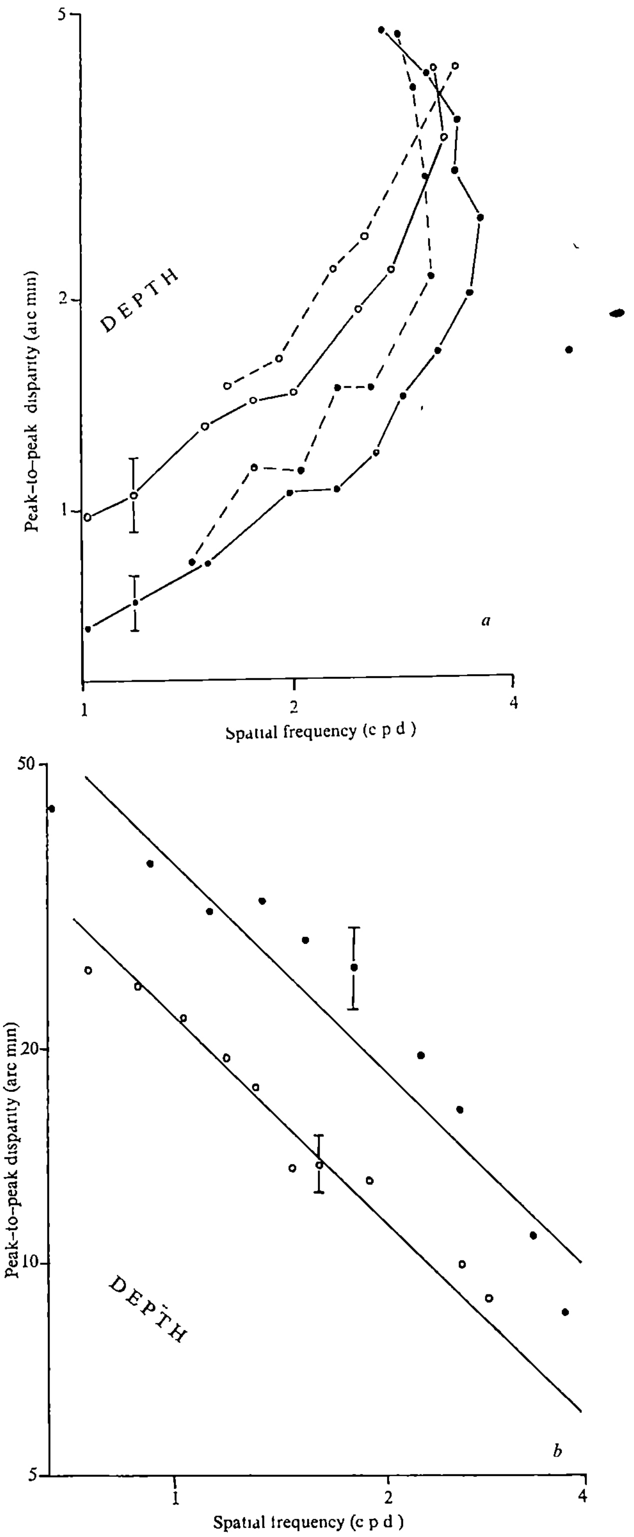

Fig. 2 Stereosensitivity for two subjects, J Z (○) and C W T (●) *a*, Region of maximum frequency, plotted in terms of peak-to-peak disparity variation versus spatial frequency of sinusoidal stereograting ——, 40% dot density, – – –, 4% dot density *b*, Region of maximum disparity in same coordinates, showing conformity to disparity scaling effect To obtain stereogratings with sufficiently low rate of change over a full range of spatial frequencies and amplitudes the gratings used were, *a*, 1–10 c p d , 0 5–5 arc min and *b*, 0 75–7 5 c p d , 5–50 arc min in spatial frequency and peak-to-peak amplitude range Both these stereogratings were produced with a dot density of 40% by area, but as a check on the effect of dot density *a* was also produced with dot density of 4% Variability of the data is shown as the average standard deviation of all pairs of readings (vertical bar above and below one point in Fig 2) In regions where the function is vertical the bar represents horizontal variability, since the direct viewing technique does not constrain the variability to one dimension

be measured by the present method For the upper depth limit, the maximum disparity (Fig 2*b*) decreases with increasing frequency, corresponding to a disparity scaling effect which conforms reasonably well with the equation (solid line in Fig 2*b*)

$$\eta = a/v$$

where η is peak-to-peak binocular disparity in arc degrees. v is spatial frequency in c p d and a is a scaling constant that has the values of approximately 0 3 for J Z and 0 6 for C W T These values may be compared with values of about 0 5 for C W T and 0 33 for another subject using line stereograms[14]

This disparity scaling equation describes a limit in rate of change of disparity across the retina This limit applies equally to global stereopsis from random-dot stereograms and qualitative stereopsis from line stereograms[18] The similarity of the scaling constant a for the two types of stimuli suggests that the disparity scaling limit is determined by the arrangement of disparity detectors in cyclopean space rather than by the difference in orientation between monocular line segments[17]

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The homeothermic status of the neonatal dog

The capacity of a warm blooded animal to increase production and conservation of body heat when it is cooled and to dissipate heat when it is warmed largely determines that range of environmental temperatures within which a stable deep body temperature can be successfully achieved

In these respects the mature dog is an example of an efficient homeotherm, able to withstand the short-term effects of ambient temperatures as low as -100° C and as high as 42° C[1-3] In contrast, the neonatal dog demonstrates a relatively poor body temperature stability and when isolated in a cooling environment it tends to adopt the temperature of that environment[2,4] This has led to an assumption that the dog is poikilothermic for some time after birth[2,5] The concept of poikilothermy in this context may have been based on a too literal interpretation of the term homeothermy Body temperature stability is the product of a number of behavioural, physiological and metabolic processes[3,6] which characterise the homeothermic state and these clearly differentiate it from poikilothermy Appreciation of the differences is of some importance in the dog as the newborn of this species is often the subject of intensive medical care requiring segregation in an environment which makes minimal metabolic demands

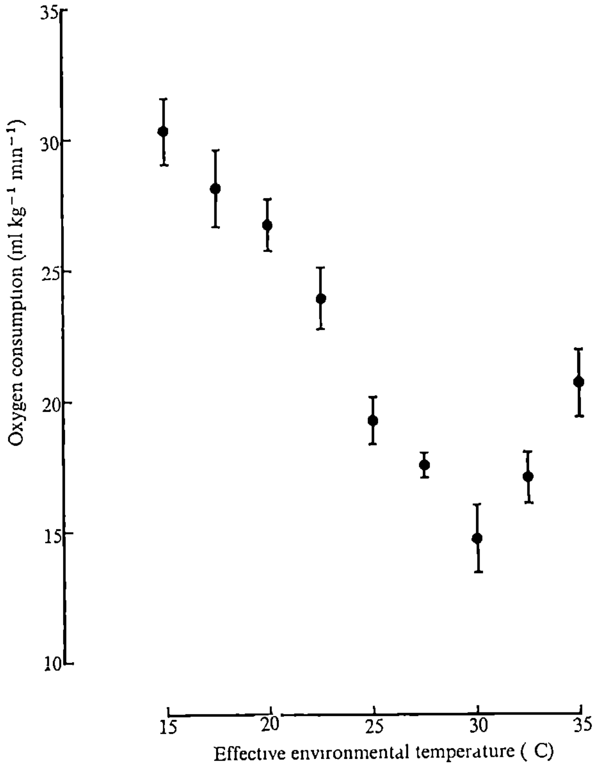

Fig. 1 Mean (± s e) oxygen consumption of groups of neonatal beagle dogs (3 d old) segregated at selected environmental temperatures for 30 min

Beagle puppies were used in this study They were housed with the bitch in insulated whelping boxes[7] in a constant ambient temperature of 28° C The core temperature of a post-prandial puppy 3 d old was recorded and it was then subjected to an interval of 30 min in an incubator at 30° C to establish equilibrium between core and surface temperatures before a period of experimental observation in an environmental chamber Nine temperatures, lying between 15° C and 35° C were used, and puppies were randomly allocated to not more than two of these temperatures on the day of observation

The environmental chamber was a Perspex cylinder surrounded by a water jacket which was incorporated into a closed air circuit, a modification of a design described earlier by other workers[8] A puppy in the chamber lay inside a plastic cylinder bearing multiple perforations to prevent bodily contact with the wall of the chamber The experimental environment was controlled in terms of air humidity (40–60%), air velocity (of the order of 1 5 cm s^{-1} surface speed) and temperature Internal wall and air temperatures were monitored by means of thermistors and the effective environmental temperature of the chamber was taken as their mean A period of 4 min was sufficient for equilibration of the apparatus after a puppy was introduced and thereafter oxygen consumption was recorded for a period of 30 min Carbon dioxide within the closed circuit was totally absorbed in a container of soda lime placed distal to the chamber in the direction of air flow Depletion of closed-circuit volume was restored by addition of fixed volume aliquots (20 ml) of pure oxygen from a float recorder which refilled automatically when empty Rate of oxygen consumption was computed as the regression coefficient of the equation relating the fixed volume of oxygen consumed to recorded times and was expressed as ml min^{-1} dry gas at s t p divided by body weight (kg) Core temperatures were recorded before and after each exposure by means of a thermistor probe inserted into the colon for a distance of approximately 6 cm Puppies were observed visually and their behaviour was recorded during periods of study Other beagle dogs 2 d old were subjected to periods of exposure in ambient temperatures between 8° and 35° C for intervals of up to 120 min Core temperatures were recorded in these instances, but not oxygen consumption.

Metabolic activity, expressed as oxygen consumption, of 3 d old puppies which were segregated in selected effective environmental temperatures for intervals of 30 min is illustrated in Fig 1 Mean oxygen consumption was minimal at an environmental temperature of 30° C Lower and higher temperatures resulted in an increase in oxygen consumption A significant difference was observed at 27 5° C ($P < 0\ 05$) and the mean rise of oxygen consumption over that range of cooling temperatures which was chosen was related to the degree of cooling stress At 15° C the mean oxygen consumption was twice that which was recorded at 30° C

Mean body weight of the group exposed to 30° C was 0 328 kg Mean oxygen consumption was 14 7 ml kg^{-1} min^{-1} Assuming that 1 ml of oxygen min^{-1} is equivalent to heat production of 7 2 kcalories/24 h (ref 8), and using a factor of 4 184 to convert kcalories to kJ (ref 6), the mean resting metabolic rate of these puppies in an environmental temperature of 30°C was 14 7 × 0 328 × 7 2 × 4 184 = 145 kJ per 24 h This compares favourably with a value of 127 kJ per 24 h which can be calculated for mammals of the same weight using the generally accepted level of 293 kJ kg$^{-3/4}$ per 24 h^{-1} for mean standard metabolic rate[6,9]

Characteristic behaviour was also observed (Table 1) At the environmental temperature at which oxygen consumption was minimal, puppies 3 d old relaxed at once and remained completely unconcerned with their surroundings At lower temperatures, a proportion of each group became alert, restless, hyperpnoeic and vocal The degree of persistence of these responses and the proportion of animals involved increased as environmental temperatures declined A similar syndrome was observed at temperatures of 32 5° C and higher Both syndromes ceased at once and the puppies resumed a state of relaxation and somnolence when they were returned to an incubator at a temperature of 30° C which appeared to be the preferred environment of puppies of this age

Core temperatures of puppies 3 d old were found on removal from the bitch to be 37 2 ± 0 4° C (mean ± s d $n = 90$) No significant differences were recorded after isolation and exposure to 30° C in an incubator or in the environmental chamber

Changes in the deep colonic temperature of these puppies are illustrated in Fig 2 If it is accepted that a mean change of colonic temperature of less than 0 5° C over a period of 30 min is indicative of thermoregulation, then puppies 3 d old were able to regulate in environmental temperatures between 20° - 30°C Exposure to temperatures outside this range resulted in deviations in body temperature which stimulated behaviour indicating that the onset of hypothermia or hyperthermia was not accepted passively (Table 1)

The influence of environmental temperatures on deep colonic temperature of puppies 2 d old after exposure for longer periods is shown in Table 2 Again, no significant changes in colonic temperatures were observed in the group exposed to 30° C in spite of a temperature differential between core and environment of 7° C A temperature differential of 12 5° C, that is an environmental temperature of 25° C, was also associated with a successful effort to regulate deep body temperatures When the initial core/environment differential was increased to 17° C degrees or more, however, puppies became hypothermic At 35°C, a core/environmental temperature differential of less than 2 5° C, hyperthermia was induced so rapidly and with such dramatic behavioural responses that observations were limited to intervals of 30 min In all instances in which deviations of deep colonic temperatures occurred, normal body temperatures were resumed after incubation at 30° C

Table 1 The behaviour of groups of beagle puppies (3 d old) segregated at selected environmental temperatures for 30 min

Effective environmental temperature (° C mean ± s e)	No of animals studied	Syndrome* Exhibited	%	95% confidence intervals[10]
35 2 ± 0 1	9	9	100	(77–100)
32 5 ± 0 1	13	5	39	(17–67)
30 1 ± 0 1	11	0	0	(0–25)
27 2 ± 0 1	14	3	21	(8–50)
25 1 ± 0 1	10	3	30	(12–62)
22 2 ± 0 2	10	7	70	(35–91)
19 9 ± 0 1	12	10	83	(60–97)
17 7 ± 0 1	8	6	75	(42–95)
14 8 ± 0 1	6	6	100	(60–100)

*Alertness, restlessness, hyperpnoea and vocalisation Zero response indicated relaxation and somnolence

These data indicate that the neonatal dog has a well delineated thermally neutral zone (TNZ) within which at rest it demonstrates a metabolic rate typical of a small mammal At an ambient temperature which is within TNZ (30° C) it maintains a stable core temperature of the order of 37° C This is similar to the mean core temperature which we have recorded in neonatal dogs of this age taken straight from the litter These animals have a nychthemeral variation of less than 2° C Already at this early age the dog is tachymetabolic and cenothermic[11]

It also displays distinctive and measurable physiological temperature regulation especially to cooling, but a relatively narrow yet clearly distinguishable tolerated ambient temperature range This range lies approximately between 20° C and 30° C (Figs 1 and 2)

The behaviour of the neonatal dog at different ambient temperatures is especially revealing (Table 1) The animal appears to

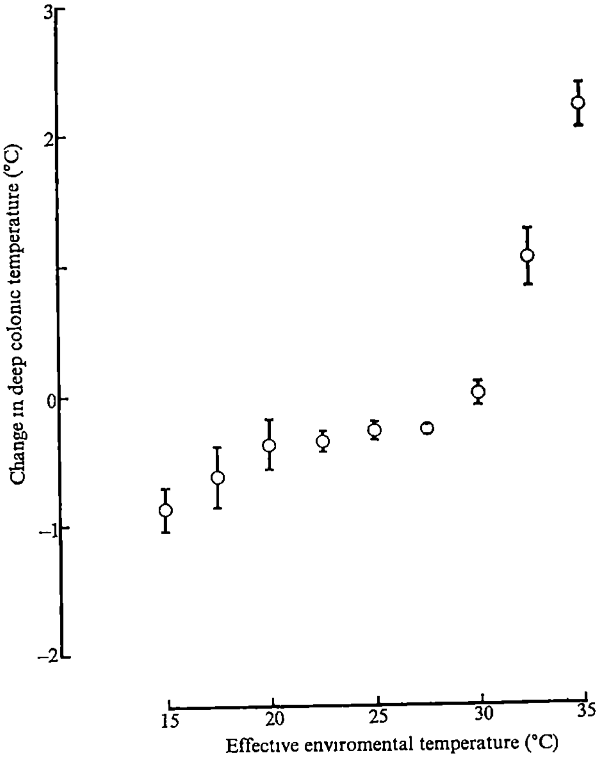

Fig 2 Mean (± s e) change in deep colonic temperatures of groups of neonatal beagle dogs (3 d old) segregated at selected environmental temperatures for 30 min

Table 2 Deep colonic temperatures of beagle puppies 2 d old on exposure to different environmental temperatures

Environmental temperature (° C)	Deep colonic temperatures (° C mean ± s e) Time (min) 0	120
35 0	37 4 ± 0 1	39 5 ± 0 2*
30 0	36 8 ± 0 2	37 1 ± 0 1
25 0	37 5 ± 0 2	36 5 ± 0 4
20 0	37 2 ± 0 3	33 7 ± 0 4
8 0	36 9 ± 0 1	28 2 ± 0 5

*30 min interval

be largely indifferent to its surroundings when it is within TNZ Within the range of tolerated ambient temperatures, in which by intrinsic means it is able to counteract the effects of thermal stress to sustain thermal balance, it demonstrates an awareness of its surroundings At ambient temperatures which mark the upper and lower limits of temperature survival and which result in progressive deviations from the preferred core temperature of the neonate, onset of hypothermia or hyperthermia is not accepted voluntarily

To consider fluctuations of core temperature in relation to ambient temperature alone and without reference to the presence of intrinsic thermoregulatory mechanisms and then go on to equate deviations of core temperature over selected ranges of ambient temperatures with a situation akin to poikilothermy may be misleading We would cite the data in Table 2 At an effective environment temperature of 35° C, far from adopting this temperature as its core temperature, the core temperature of the neonatal dog rises dramatically It is the presence rather than the efficacy of physiological temperature regulation which could well be the final arbiter when taxonomical difficulties are encountered

Temperature regulation of the neonatal dog is admittedly very much more restricted compared with the adult, but this may be regarded as simply an expression of an immature phase in the development of what later becomes a highly efficient form of body temperature homeostasis in this species We submit that our data go some way towards fulfilling the essential criteria by which one should judge a homeotherm and we conclude that the neonatal dog is an immature homeotherm, thus rejecting the hypothesis that it could be described as poikilothermic at this age

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Cyclic AMP levels in *Phycomyces* during a response to light

One of the many metabolic functions attributed to adenosine 3′ 5′-cyclic monophosphate (cyclic AMP) is that of a mediator in the responses of living organisms to light stimulation It has been associated with dopaminergic synaptic activities and also with the primary visual process in the retina In the rod outer segments of the frog, *Rana pipiens*, cyclic AMP concentrations are diminished by illumination Regulation is through the cyclic nucleotide phosphodiesterase[1] In addition, an adenyl cyclase has been isolated from retina which is activated by the catecholamine dopamine, dopamine is thought to be an important neurotransmitter in the retina[2] The exact relationship between the initial transduction of the light signal and the attenuation in the levels of cyclic AMP is unknown One reason for this is the complexity of the tissue and the difficulty of isolating clean fractions I describe here a simpler system in which levels of cyclic AMP decrease in response to light stimulation much as in retina and where all the transduction occurs within a single cell

The sporangiophore of the fungus *Phycomyces blakesleeanus* is a giant single cell—several cm long and 100 μm in diameter—which responds in minutes to light, gravity, touch and the proximity of objects, the last being termed its autochemotropism (formerly avoidance) In a mature sporangiophore (stage IVb), the sensitive area is identical to the growing zone, a region 3 mm long, just below the apical sporangium, where essentially all elongation occurs[3] In the case of the response to light, the nature of the behaviour has been worked out in detail using an automatic tracking system[4], and several mutants have been isolated[5] Figure 1 illustrates an example of a growth response to an increase in light intensity The response is transient with a latency of about 3 5 min, maximal at 4–5 min and essentially complete after 12 min The noise has been shown to be endogenous and to be associated with growth regulation after the reception of the light signal[4] However, in spite of some initial efforts[6], no chemical or morphological events have been shown to accompany or precede the observed acceleration of growth I have investigated the levels of cyclic AMP in *Phycomyces* sporangiophores and found a sharp decrease well before the observed growth response

P blakesleeanus, NRRL1555, was grown on 4% potato dextrose agar supplemented with 0 1% yeast extract Synchrony of sporangiophore development was achieved by a transfer from dark to light The mature sporangiophores (3 5±0 2 cm) were dark adapted for 40 min At time zero they were exposed to 3 μW cm^{-2} continuous fluorescent light At intervals 1 g of sporangiophores was plucked, quick frozen in liquid nitrogen and ground with a pestle and mortar The fine powder was rehomogenised in perchloric acid and the supernatant liquid, after precipitation of proteins and KClO$_4$, was subjected to anionic exchange chromatography A tracer of approximately 8,000 c p m ^{3}H-cyclic AMP was added at the homogenisation step to aid observation of the elution profile and assessment of final recovery Recoveries varied from 75 to 95% However, only the middle 40% of the cyclic AMP peak eluted from the column was pooled, concentrated and assayed Final results were corrected for recovery and subfractionation I used the assay of Brown *et al*[7] which is a modification of the competitive binding protein assay of Gilman[8] The amounts of cyclic AMP found in dark adapted sporangiophores varied from 0 1 to 0 3 nmol g^{-1} of wet weight Figure 2 illustrates the normalised

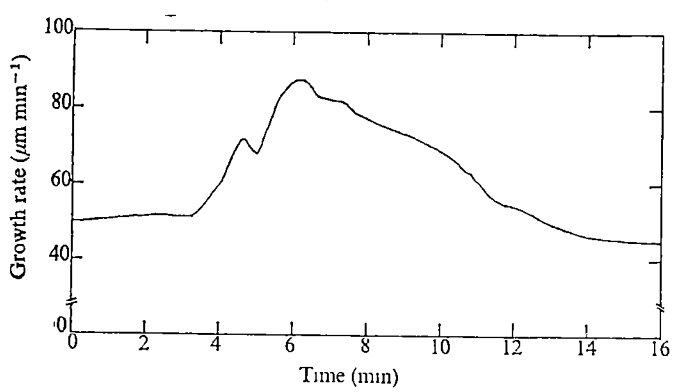

Fig 1 A typical growth response of *Phycomyces blakesleeanus* to an increase of light intensity at 24° C Conditions are comparable with those used to assay cyclic AMP The adapting light intensity was in the normal range, less than about 20 μW cm^{-2} and the final light intensity was sufficient to saturate the response system The step-up was imposed at time zero The latency was 3 5 min

results of assays for cyclic AMP during a response to continuous light

Cyclic AMP levels decreased precipitously to about 50% of the initial dark value within 1 min of light stimulation, well before the appearance of a growth response Indeed the level had returned to approximately the preillumination value at about the time that growth was first observed This is the first direct *in vivo* demonstration of a change in cyclic AMP level in a responsive system after light stimulation The sharp decrease in cyclic AMP suggests that the light receptor for *Phycomyces* is a nucleotide cyclase or possibly a cyclic mononucleotide phosphodiesterase, or may be closely associated with one of these enzymes such that light inhibits adenyl cyclase or activates phosphodiesterase Thus the primary transduction event may be the attenuation of cyclic AMP concentration Goridis and Virmaux[9] hypothesised that attenuation of phosphodiesterase is the general mechanism for the function of all sensory receptors

Because the slight decrease in cyclic AMP during the transient growth response was unexpected, the investigation was extended beyond the response (Fig 2) I found that levels of cyclic AMP had stabilised after the response, the levels in continuous light were perhaps slightly lower in these experiments than during continuous darkness

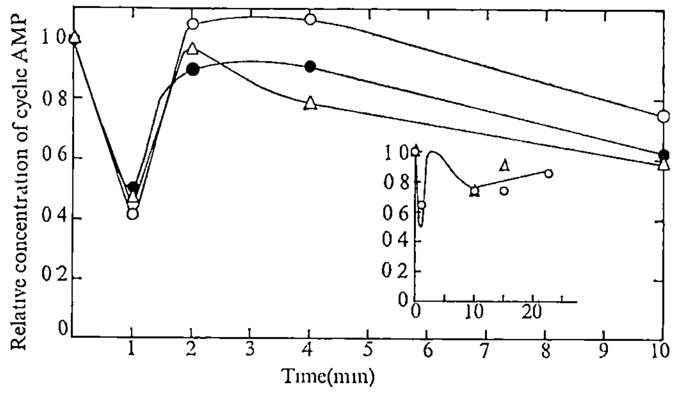

Fig 2 The normalised level of cyclic AMP in stage IVb sporangiophore as a function of the time after a light step-up at time zero The curves refer to three separate runs, each point being the mean average of five to eight determinations The absolute amounts of cyclic AMP initially present were ○, 150, ●, 302, △, 113 pmol g^{-1} wet weight Standard deviations of the mean ranged from 10 to 15% Temperature was 24° C The amount of protein in these sporangiophores was 5 0 mg g^{-1} wet weight as measured by the method of Lowry Details of the procedure are given in the text The inset shows levels of cyclic AMP in stage IVb sporangiophores extended to 25 min after the increase Other details are as in the rest of the figure The absolute amounts of cyclic AMP initially present were ○, 273 and △, 282 pmol g^{-1} wet weight

Because levels of possible interfering substances have not been investigated thoroughly in fungal material, I was careful to eliminate factors other than cyclic AMP that might affect my measurements The validity of the reported estimates is supported by (*a*) the careful ion-exchange column isolation, (*b*) the fact that when isolate was subjected to prolonged treatment with rat brain phosphodiesterase[10], boiled and reassayed, no cyclic AMP was found, while in a parallel experiment without this treatment the usual amount was found, and (*c*) several dilutions of cyclic AMP isolate yielded identical initial concentrations

Preliminary investigations indicate a very high adenyl cyclase activity consistent with high levels of product Cytoplasm from *Phycomyces* sporangiophores grown and assayed in continuous light has a V_{max} of 1 3 nmol of cyclic AMP produced per min per mg of protein and a high affinity for ATP (K_m=0 5 mM) as assayed by a slight modification of the method of Bar and Hechter[11]

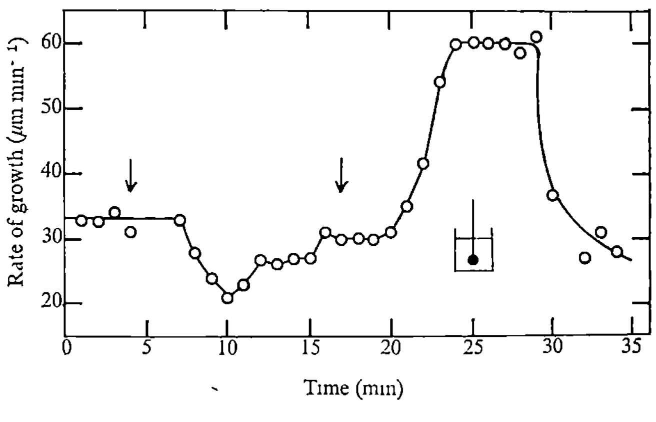

Fig 3 The response to the addition of dibutryl cyclic AMP (indicated by left hand arrow) to a growing sporangiophore The sporangiophore was initially suspended in fluorocarbon so that the growing zone was submerged as illustrated The nucleotide was added with careful stirring so as to make a final concentration of 1 mg ml^{-1} in 1 4 ml The reproducibility of the response was quite good—in five separate measurements the latency was 3 5 min and the mean decrease at the minimum was 32%±3% (s d) The growth response to a bilateral blue light stimulation (at right hand arrow) was normal in the presence of dibutyryl cyclic AMP, (0 25% Triton X-100 was present to solubilise the nucleotide Neither this nor stirring alone caused any discernable changes in growth rate)

Adenyl cyclase has been localised cytochemically using the specific inhibitor adenylyl imidodiphosphate by a method developed for islets of Langerhans[12] Cyclase is abundant in the growing zone of the sporangiophore and is not present in the nongrowing zone areas The plasmalemma, mitochondria and cytosol all have significant activity (my observations with C J M Farnham)

One might expect then that exposure to cyclic AMP would induce a transient negative growth response The sporangiophore of *Phycomyces* grows poorly under water but well in the fluorocarbon perfluorotributylamine In such a system, chemicals can be added and the response of the sporangiophore observed The introduction of dibutyryl cyclic AMP at a final concentration of 1 mg ml^{-1} consistently leads to a transient 30% decrease in the rate of growth (Fig 3) indistinguishable from the response to the dimming of light Results obtained with the phosphodiesterase inhibitor theophylline are similar This again is consonant with the hypothesis that in *Phycomyces*, modulation of cyclic AMP concentration is an important link between the reception of light and the observed transient changes in growth rate

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Glucocorticoids and hCG sensitivity of rat testicular Leydig cell

THERE is unequivocal evidence that the foetal Leydig cell in the rat is under the control of gonadotrophins[1] whereas the postnatal Leydig cell is insensitive to hypophyseal luteinising hormone (LH) (ref 2) as well as to exogenous human chorionic gonadotrophin (hCG) (refs 3 and 4) We have shown recently that the hCG insensitivity of the Leydig cell during the first 2 weeks after birth is not due to the lack of the specific hCG receptor nor of an enzyme adenyl cyclase, both of which are prerequisites of hCG action[3,4] The receptor is a constitutive protein which is already present in the foetal Leydig cell[3]

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It seems obvious that the apparent loss of hCG sensitivity of the Leydig cell after birth must be causally connected with the separation of the foetus from the mother It seems likely that the cells are deprived of a stimulus for growth and activity This stimulus might be another hormone which is present in the rat foetus, absent after birth and simultaneously restored with the onset of hCG sensitivity 2 weeks later The glucocorticoids of the adrenal cortex fulfil such a requirement The plasma corticosterone level is high before and shortly after birth[6], decreases to a very low level in the 12 d old rat[7] and increases again afterwards[8] Therefore we have tested whether hCG sensitivity during the insensitive period is inducible by hydrocortisone and adrenocorticotrophic hormone (ACTH) respectively Alcohol dehydrogenase (ADH, E C 1 1 1 1), an enzyme specifically located in the Leydig cell was used as a marker for Leydig cell function

Rats of strain SIV-50 were used throughout the experiments For the study of prenatal animals at a definite developmental stage, mating was determined by examining the presence of copulation plugs in the female rats (day of vaginal plug=day 1 of pregnancy) At the respective experimental days of pregnancy the animals were killed, exsanguinated and the testes of the foetuses were removed Varying numbers of animals were used in each experiment dependent on the age of animals: 20-40 foetuses, 15-20 animals between birth and day 8, 10 animals between days 9 and 13, five animals between days 14 and 16 and one animal between days 25 and 50 Each experiment was done at least in triplicate, if not otherwise stated The procedures used for the preparation of the animals and for ADH activity measurements were similar to those described earlier[3].

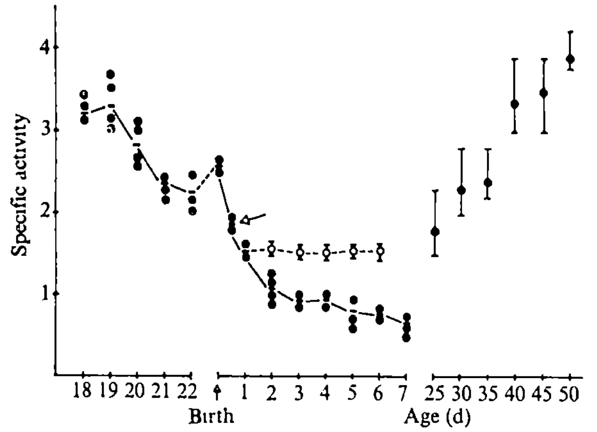

Fig. 1 Enzyme activity of alcohol dehydrogenase (mU mg^{-1} protein) in the testis of foetal and postnatal control rats and in chronically hydrocortisone treated rats Hydrocortisone acetate was purchased as Hydrocortison in a watery suspension (25 mg ml^{-1}) from Hoechst AG, Frankfurt, West Germany ●, Controls, arrow, controls 8 h after birth (each circle represents one experiment), ○ animals treated daily with 1 25 mg hydrocortisone subcutaneously from the first day after birth till to day 6 of life Preparation of the testes was performed 7 h after the last injection In hydrocortisone-treated rats and in control rats between days 25 and 50 of age at least three experiments were performed The main values and deviations are included.

As shown in Fig 1 in the day 19 foetal rat testis ADH activity is as high as in the 45 d old rat when spermatogenesis is fully established During late foetal development testicular ADH activity decreases, but the most dramatic decrease occurs during the first 2 d after birth, the lowest activity is found at 11 d of age[4] Daily injections of 1 25 mg hydrocortisone from day 1 after birth onwards till day 6 of life prevent the decrease in ADH activity Furthermore, by daily injections of 80 IU hCG in addition to hydrocortisone even the foetal enzyme activity level is restored (not shown)

We have reported recently that the postnatal Leydig cell, in spite of an intact apparatus for the response to hCG, is insensitive to the hormone during the first 2 weeks after birth[3,4] Figure 2 shows that the cell becomes sensitive to gonadotrophins after hydrocortisone or ACTH treatment After a single injection of hydrocortisone and hCG simultaneously, on each experimental day, on testicular ADH activity increases to a greater extent than after the injection of hydrocortisone alone

Figure 3 shows that a single simultaneous injection of 2 5 mg of hydrocortisone and 80 IU hCG into rats at day 6 and day 8 after birth results in a maximum increase of testicular ADH activity 31 h later Afterwards the activity decreases, and reaches nearby control levels 80-100 h after the injection Since exogenous hCG cannot stimulate ADH activity during this developmental period the decrease must be due to hydrocortisone degradation during the experimental time interval These results indicate that an adequate glucocorticoid level has to be maintained in order to guarantee hCG responsiveness of the Leydig cell As a nearly adult plasma corticosterone level is achieved in the 21-d-old male rat[8], hydrocortisone injection into rats older than 21 d should be ineffective Accordingly, testicular ADH activity in hydrocortisone-treated 26-d-old (5 0 mg per animal) and adult animals (12 5 mg per animal) corresponds to control animals 24, 31, 58, 72 and 96 h after the injection (not shown)

From our results we infer that the insensitivity of the postnatal Leydig cell to gonadotrophins is due to the lack of glucocorticoids during this developmental period The foetal adrenal cortex begins to secrete glucocorticoids on gestational day 16 or 17 (ref 9) and could therefore provide the natural stimulus for the LH sensitivity of the foetal Leydig cell The maximal concentration of corticosterone in the plasma and adrenal glands occurs between days 18 and 19 and then decreases till birth[6] The hypothalamic control of the pituitary–adrenal system begins to operate between days 18 and 22 of gestation[10] After birth, however, the pituitary–adrenal axis becomes dormant and is re-established approximately 2 weeks later, the normal control of ACTH release emerges from day 11 to maturity[11] Our ADH activity measurements in foetal and postnatal testicular tissue, as well as after hydrocortisone or ACTH treatment, are in line with these findings

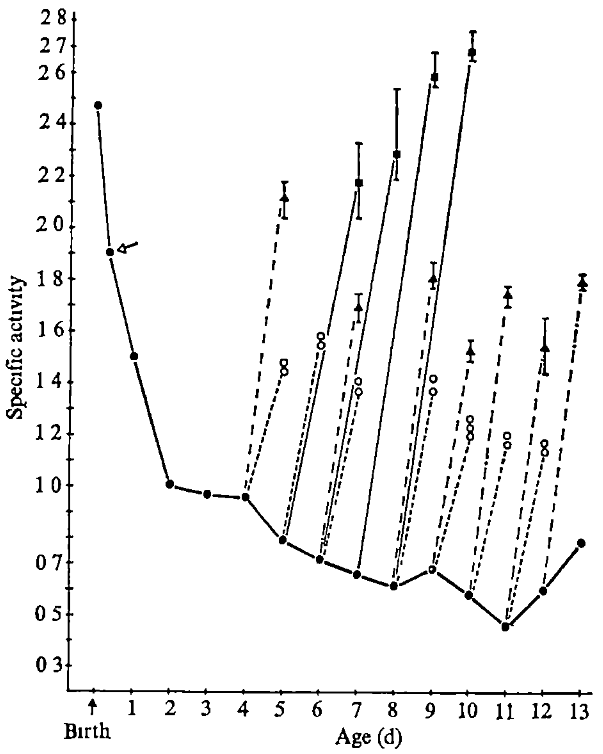

Fig. 2 Enzyme activity of alcohol dehydrogenase (mU mg^{-1} protein) in the testis of rats between birth and the day 13 of age in controls and treated animals ●, Controls, →, controls 8 h after birth (for further details see Engel and Frowein[4]), ○, animals treated with 2 5 mg hydrocortisone subcutaneously and prepared 31 h later Each circle represents one experiment ▲, Animals were injected simultaneously with 2 5 mg hydrocortisone intraperitoneally and 80 IU hCG, subcutaneously, dissolved in 0 1 ml of a 0 9% NaCl solution (preparation of the testes was done 31 h after the injections) ■, After two doses of 1 IU ACTH (dissolved in 0 1 ml of a 0 9% NaCl solution) subcutaneously at a 24 h interval The testes were removed 7 h after the last injection Mean values and deviations resulting from at least three experiments with hCG+hydrocortisone (▲) and ACTH (■) are demonstrated HCG was supplied as Primogonyl (crude hCG preparation, only hCG activity, specific activity of 3,000 IU mg^{-1} protein) from Schering AG, Berlin, West Germany Two ACTH preparations were used Depot-Acethropan (highly purified preparation from animal hypophysis, salt-like bound to polyphlerotine phosphate, exclusive ACTH activity, specific activity of 200 IU mg^{-1} protein) from Hoechst AG, Frankfurt, Western Germany and Depot-Synacthen (synthetic ACTH preparation, β^{1-24} corticotrophin hexa-acetate, the first 24 amino acids are identical to the genuine human ACTH) from CIBA-Geigy AG, Wehr, Western Germany The results obtained with both ACTH preparations were identical.

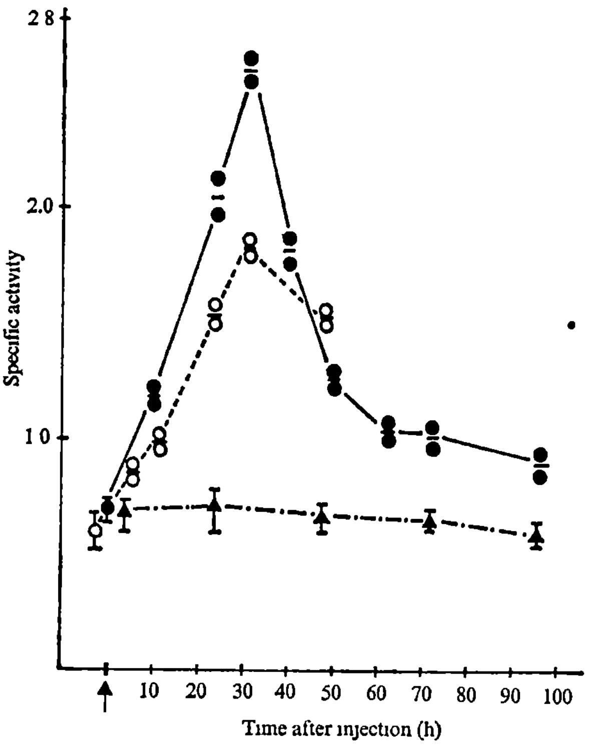

Fig. 3 Enzyme activity of alcohol dehydrogenase (mU × mg^{-1} protein) in the testis of rats at day 6 (●) and day 8 (○) of life, prepared between 5 and 98 h after a single simultaneous injection (→) of 2 5 mg hydrocortisone (intraperitoneally) and 80 IU hCG (dissolved in 0 1 ml 0 9% NaCl solution) subcutaneously Each circle represents one experiment Also ADH activities in rats between days 6 and 10 of age, daily treated with hCG, subcutaneously, from birth onwards (▲) are included in the figure, for further detail see Engel and Frowein[4].

Obviously, the adrenal glucocorticoids are necessary for the initiation of the sexual maturation process in the male rat Recently Gorski and Lawton[12] and Ramaley[13] have provided evidence that the adrenals play a definite role in determining the time of onset of puberty in the female rat In immature or adult male rats cortisone has been reported to stimulate testis weight and to effect testicular morphology (for review see Gomes[14]) Zondek and Zondek[15] found a positive correlation between hypoplasia of the adrenal cortex and the number of Leydig cells in anencephalic human foetuses Brook *et al*[16] reported a 15 9-yr-old boy with congenital adrenal hypoplasia exhibiting no signs of puberty. Furthermore, in men with Addison's disease Leydig cells seem to be sensitive to hCG only to a very small extent (E Nieschlag, unpublished) ACTH injections into prepubertal children result in a significant increase of plasma testosterone and dehydroepiandrosterone concentration[17]

The question arises by which mechanism glucocorticoids may provoke hCG sensitivity of the Leydig cell We have shown recently that hydrocortisone-1-2-^{3}H can bind to a cytoplasmic receptor in rat testicular tissue (W E , and J F , unpublished) In contrast to liver, however, the hydrocortisone receptor complex is not translocated to the nucleus (J F and W E , unpublished) Since hydrocortisone has a similar effect on ADH activity in the hCG insensitive testis as theophylline[3,4], we assume that glucocorticoids act by alteration of cyclic 3 , 5′-monophosphate phosphodiesterase activity in the Leydig cell We already have experimental evidence supporting this view (W E , and J F , unpublished) A similar effect of the glucocorticoids has been described in various other tissues[18–20].

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Indomethacin potentiates PGE₁ stimulated cyclic AMP accumulation in human synoviocytes

PROSTAGLANDINS have both inhibitory and stimulatory effects on inflammatory and immune processes[1] The capacity of the nonsteroidal anti-inflammatory agents, aspirin and indomethacin (IM), to inhibit the synthesis of certain prostaglandins emphasises the potential importance of this hormone system in the pathogenesis and treatment of inflammatory arthritis[2] Since the prostaglandins inhibit certain cellular functions involved in the production of some forms of arthritis[3], suppress the inflammatory response of adjuvant arthritis[4-7] and yet induce an acute inflammatory response when injected into the dog stifle joint[8], there remain certain paradoxes with regard to the role of prostaglandins in inflammatory joint diseases.

We have used human synoviocytes in culture to investigate directly the effects of IM on these cells in the presence and absence of prostaglandins We report here results which demonstrate a significant increase in the intracellular concentration of cyclic AMP when human synoviocytes in culture are treated with PGE_1. Our results also show this hormone-mediated effect to be potentiated by IM, an inhibitor of human synoviocyte cyclic adenosine 3′,5′-nucleotide phosphodiesterase (PDE) activity

Human synovial lining (SLC) or tendon sheath lining (TLC) cells were dissected from underlying tissue at surgery by using a dissecting microscope, cut into small pieces and planted in growth media. When sufficient outgrowth was observed, cultures were trypsinised and subcultured for experimental use, Subcultured cells showed ultrastructural characteristics similar to those published for human type A and B synoviocytes in tissue culture[9,10] and produced significant amounts of hyaluronate using a qualitative mucin test[11]. Cells were routinely split 1 to 2 h after the first subculture had reached confluency, thus, the passage number roughly corresponds to the number of cell doublings after the first subculture. Cells were used between the fourth and twelfth passage and were mycoplasma negative Protein was measured by the Lowry method[12], and the Thompson and Appleman method[13] was used to determine PDE activity ^{3}H-cyclic AMP (28 Ci mmol^{-1}, Schwarz-Mann) was purified by thin layer chromatography[13]

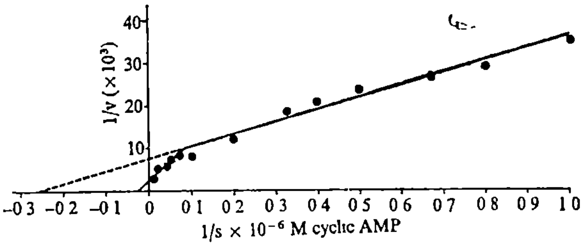

Fig. 1 Lineweaver-Burk plot of synoviocyte cyclic adenosine 3′, 5′–monophosphate phosphodiesterase activity Each point represents the average of two determinations, and the slope and intercept calculations are by linear least square analysis The maximal velocities for the low and high K_m plot are 135 and 435 pmol cyclic AMP hydrolysed per 30 min, respectively

The control values for cyclic AMP in the experiments presented (Table 1) are somewhat higher than have been determined for other nonestablished cell lines When human synoviocytes were assayed without a 1 h preincubation in serum-free media, however, we found the cyclic AMP concentration to be 18 9 ± 2 66 pmol per mg protein which is similar to the concentrations found by others in quiescent fibroblasts[14] The absence of serum for 1 h results in a two to threefold increase in intracellular cyclic AMP concentrations which agrees with the results of Froehlich and Rachmeler[15] who found an inverse relationship between intracellular cyclic AMP and media serum concentrations for human diploid fibroblasts Ethanol at concentrations up to 0 53% had no significant effect on the intracellular cyclic AMP concentrations.

IM used alone at concentrations of 10^{-4} and 10^{-5} M had little effect on intracellular cyclic AMP concentration (Table 1, experiments 1 and 2) IM at 10^{-6} M resulted in a modest decrease in cyclic AMP concentration (Table 1, experiment 1) but, the addition of PGE_1 plus IM (10^{-4} to 10^{-6} M) routinely caused a potentiation of the PGE_1 response in both TLC and SLC cell lines IM added to the cells in increasing concentrations resulted in a concentration-dependent increase in intracellular cyclic AMP over the PGE_1-stimulated levels (Table 1, column 4) The potentiation of the PGE_1 effect on intact cells occurs at concentrations of IM (10^{-5} M) known to give effective anti-inflammatory responses *in vivo* Our data (Table 1, experiment 1) demonstrating a modest decrease in intracellular cyclic AMP concentrations with very low concentrations of IM alone (10^{-6} M) suggest a mechanism involving prostaglandin synthetase (PS) inhibition At high IM concentrations, however, both PS and PDE activities might be inhibited resulting in little change in cyclic AMP The PGE_1-dependent effect of IM may be the result of an effect of IM on either cyclic AMP or PGE_1 metabolism or an effect of PGE_1 on IM metabolism, The observations that theophylline, an inhibitor of PDE activity, also potentiated the PGE_1-responsiveness of human synoviocytes (Table 1, experiment 4) and that IM is an inhibitor of epiphyseal cartilage PDE activity[19] led us to examine the effect of IM on synoviocyte PDE activity

TLC homogenates demonstrated linear PDE activity up to protein concentrations of 185 μg ml^{-1} using 1 × 10^{-6} and 1 × 10^{-4} M cyclic AMP, respectively Kinetic studies (Fig. 1), using conditions in which less than 20% of the substrate was converted to product, provided data that gave nonlinear Lineweaver-Burk plots with two apparent Michaelis constants for cyclic AMP (4 0 × 10^{-5} M and 3 9 × 10^{-6} M) Indomethacin at a final concentration of 10^{-3} M resulted in 89% inhibition of cyclic AMP–PDE activity with 1 × 10^{-6} M cyclic AMP (Table 2) and only 10 8% inhibition with 1 × 10^{-4} M cyclic AMP as substrate The concentration of IM causing 50% PDE inhibition was 1 35 × 10^{-4} M Significant inhibition of PDE activity with low and not with high substrate concentrations demonstrates a specificity for the low K_m enzyme which is presumed to represent the enzyme species most important for the physiological control of intracellular cyclic AMP concentrations The concentrations of IM to obtain 50% inhibition of the PDE activity do not reflect therapeutic concentrations of the drug Since the lysosomal system, however, accounts for the cellular uptake of indomethacin[21], intracellular concentrations may be higher than the serum concentrations of IM stated as effective for therapeutic action In addition, the loss of cell membrane integrity which results in altered receptors, transport, and concentrating mechanisms in broken cell preparations makes this assay a less competent system for studying physiological PDE activity

Table 1 The effect of PGE$_1$ and indomethacin (IM) on intracellular cyclic AMP concentration

Cell type		Cyclic AMP pmol per mg cell protein	Increment in cyclic AMP concentration above PGE$_1$–stimulated level (%)
TLC (Experiment 1)	Control (0 2% ethanol)	103 0± 18 2	
	IM (10^{-4} M)	84 0± 11 0	
	IM (10^{-5} M)	87 3± 21 4	
	IM (10^{-6} M)	68 4± 22 4	
SLC (Experiment 2)	Control (0 2% ethanol)	56 7± 19 4	
	PGE$_1$	468 6± 45 4	
	IM (10^{-4} M)	92 7± 17 8	
	PGE$_1$+IM (10^{-4} M)	869 1±107 1	85 47
TLC (Experiment 3)	Control (0 2% ethanol)	74 3± 7 0	
	PGE$_1$	1,423 7± 40 5	
	PGE$_1$+IM (10^{-5} M)	2,009 8± 57 2	41 18
	PGE$_1$+IM (10^{-6} M)	1,690 1± 98 6	18 76
SLC (Experiment 4)	Control (0 53% ethanol)	60 5± 5 2	
	PGE$_1$	156 2± 39 3	
	PGE$_1$+7-oxa-13-prostynoic acid (50 µg ml^{-1})	79 5± 24 4	
	PGE$_1$+theophylline (10^{-3} M)	848 6±175 2	
	PGF$_{1\alpha}$	38 8± 8 3	

Human synoviocytes isolated from either tendon (TLC) or synovial (SLC) linings were planted in 60×15 mm plastic culture dishes and grown to confluency using BME (Gibco) supplemented with 20% foetal calf serum, 2 mM L-glutamine, 100 units ml^{-1} penicillin and 100 µg ml^{-1} streptomycin Following a 60 min preincubation at 37° C in serum-free media, the cells were treated with either indomethacin at a final concentration between 10^{-4} and 10^{-6} M, theophylline at 10^{-3} M or an equal volume of 100% ethanol for 15 min Subsequently the cells were treated for another 15 min with either PGE$_1$ (3 µg ml^{-1}), PGF$_{1\alpha}$ (3 µg ml^{-1}) or an equal volume of 100% ethanol 7-oxa-13-prostynoic acid (50 µg ml^{-1}) was added in appropriate incubations together with PGE$_1$ All monolayers were processed by the method of Manganiello and Vaughan[16] Cyclic AMP was assayed by the protein-binding method of Gilman[17] All test compounds were solubilised in small volumes of 100% ethanol except for theophylline which was added in BME All incubations were done on duplicate plates and cyclic AMP assayed at two dilutions Appropriate control samples were assayed for cyclic AMP after the addition of purified beef heart PDE to determine the amount of nonspecific binding[18] All values shown have been corrected for this nonspecific binding Each cyclic AMP concentration given represents the mean± s d of four determinations Final concentrations in the incubation are given for each treatment.

These results and others showing that IM causes an increment in cyclic AMP in the presence of PGE$_1$ receptor-site saturation (D S N and C P C unpublished) suggest that the IM potentiation of PGE$_1$ stimulation of cyclic AMP is most likely the result of PDE inhibition

In addition to the IM effect on cyclic AMP, our experiments (Table 1) demonstrate a direct effect of PGE$_1$ on human synoviocytes PGE$_1$ increased the intracellular cyclic AMP concentration in human synoviocytes derived from both tendon sheath and synovial membrane

The specificity of the PGE$_1$ response is supported by the fact that PGF$_{1\alpha}$ did not cause an increase in intracellular synoviocyte cyclic AMP concentration, and that 7-oxa-13-prostynoic acid, a competitive inhibitor of prostaglandin binding in some systems[22], depressed PGE$_1$ responsiveness (Table 1, experiment 4)

Our results show that IM potentiates the effect of PGE$_1$ on intracellular cyclic AMP concentration by inhibiting synoviocyte PDE activity Since prostaglandins are found in inflammatory exudates[1], the PGE$_1$ effect amplified by IM may represent a new component of the anti-inflammatory effect of this drug We have also shown a PGE$_1$-mediated stimulation of human synoviocytes The effects of PGE$_1$ and IM on intracellular cyclic AMP concentration suggests that, as has been shown for other tissues[23], the inflammatory and immune responses of human synoviocytes may be regulated by this mechanism.

Table 2 Inhibition of human synoviocyte cyclic nucleotide phosphodiesterase activity by indomethacin

Indomethacin concentration (M)	PDE specific activity (units per mg protein) 10^{-6} M cyclic AMP as substrate	Inhibition (%)
Control	543	—
10^{-3}	61	89
10^{-4}	389	28
10^{-5}	516	5
10^{-6}	525	3

Monolayers were washed five times in homogenising buffer (0 04 M Tris– HCl, *p*H 8 0 containing 10 9% sucrose) at 4° C All homogenates were prepared by the method of D'Armiento *et al*[20], and 25×10^{-6} cells per 7 5 ml of homogenising buffer resulted in a protein concentration of 0 775 mg ml^{-1} One unit of PDE activity was defined as the amount of enzyme required to hydrolyse 1 pmol cyclic AMP per 30 min at 30° C Indomethacin was solubilised in DMSO, and control and experimental incubations contained in final concentration 1 25% DMSO Appropriate control incubations established that the observed effects of IM on PDE activity were not the result of 5′–nucleotidase inhibition All assays were run in triplicate

These investigations establish a role for prostaglandins and cyclic AMP in the biological function of human synoviocytes and propose a new mechanism for the anti-inflammatory activity of indomethacin

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Blockage of cation permeability across the tight junctions of gallbladder and other leaky epithelia

THE main route of passive ion permeability in gallbladder and other 'leaky' epithelia is through the so-called 'tight' junctions between cells, not through the cells themselves[1] I report here that the protonated form of the organic base 2,4,6-triaminopyrimidine (TAP), reversibly and specifically blocks cation permeation across this tight junction route in frog gallbladder and several other leaky epithelia Since in these epithelia the tight junction permeability is mainly (or even completely) cationic, in principle on addition of TAP a low resistance, leaky epithelium can become a high resistance epithelium

Transepithelial electrical potential differences (p d) and conductances (G) were measured across flat sheets of bullfrog gallbladder mounted between two well-stirred chambers at 23° C The experimental procedure, and methods for obtaining ionic permeability (P_{II}) and conductance (G_I) values (correcting G_{Na} for a small leakage current) were described previously[2] The rate of fluid transport was measured in 'sac' preparations of bullfrog gallbladder by the gravimetric procedure described in detail by Diamond[3] Since 2,4,6-triaminopyrimidine (pK_a = 6 72) is only active in the protonated form (manuscript in preparation), all the experiments were done at pH 6 0, and only concentrations of the protonated form are reported TAP was effective when added to the mucosal, serosal, or both solutions, but in the experiments reported here it was added symmetrically to both sides of the preparation

Addition of TAP invariably increases the membrane resistance (R_m) and decreases the P_{Na}/P_{Cl} ratio Both effects are totally due to a specific decrease in G_{Na} Let us define the fractional inhibition (α) of the partial conductance of an ion X as $\alpha_X \equiv 1 - [G_X\ (\text{TAP})/G_X\ (\text{control})]$, where G_X (control) and G_X (TAP) are the conductances of the ion species X in the control period and in the presence of TAP respectively Each point of Fig 1*b* represents an experimental α_{Na} determination in an individual frog gallbladder at a given TAP concentration The figure shows that as the TAP concentration increases α_{Na} approaches 1, that is, G_{Na} is reduced At 19 mM 92% of G_{Na} is inhibited The dependence of α_{Na} on TAP concentration suggests a saturation kinetics Therefore, the experimental values were fitted by the equation

$$\alpha_{Na} = C\,[\alpha_{Na}\ (\max)]/[K_m + C] \qquad (1)$$

where C represents the concentration of TAP, and α_{Na} (max) and K_m are two constant parameters The curve drawn through the experimental α_{Na} values of Fig 1*b* is based on the least mean squares fit of the experimental values to equation 1 The fitted parameters and their standard deviation are K_m = 2 6 ± 0 1 mM, and α_{Na} (max) = 1 0 ± 0 0 This indicates that 100% of G_{Na} can be inhibited by TAP at sufficiently high concentrations, and that the inhibitory process can be described by a single constant

The situation for G_{Cl}, illustrated in Fig 1*a*, is very different at all TAP concentrations tested $\alpha_{Cl} \simeq 0$, meaning that TAP does not affect G_{Cl} This indicates that Na^+ and Cl^- migrate through separate pathways, and is in agreement with previous evidence[4] suggesting that the Cl^- pathway is a relatively small ($G_{Cl}/G_{Na} \simeq$ 0 2 in untreated frog gallbladder) parallel leakage or free solution shunt

In five other leaky epithelia[1] (six rabbit gallbladders, two *Necturus* gallbladders, two rabbit small intestines, two bullfrog small intestines, and two bullfrog choroid plexuses) TAP had qualitatively the same effect as in frog gallbladder In all cases TAP increased R_m and decreased P_{Na}/P_{Cl}, and the effect could be completely attributed to an inhibition of G_{Na}

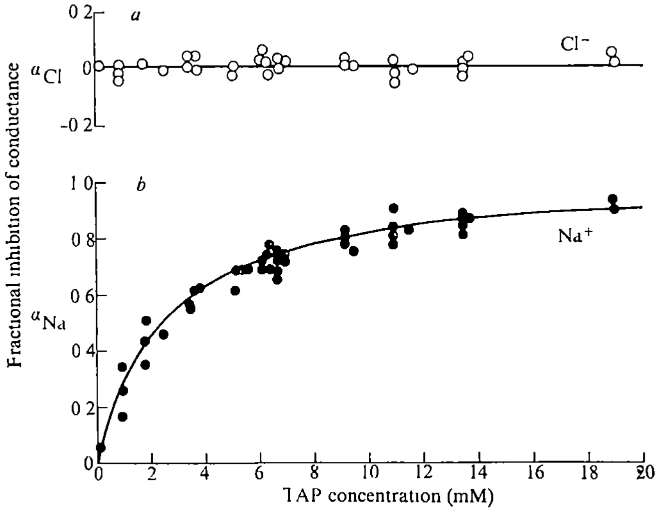

Fig 1 Effect of TAP on G_{Na} and G_{Cl} The fractional inhibition of G_{Na} (●, α_{Na}, below) and G_{Cl} (○, α_{Cl}, above) are plotted against TAP concentration Each point corresponds to one experimental value Notice that G_{Na} is strongly inhibited (α_{Na} increases) as TAP concentration increases, whereas G_{Cl} is unaffected The line drawn through the α_{Na} values is the least mean squares fit to equation 1 yielding K_m = 2 6 mM, α_{Na} (max) = 1 0 The line through the G_{Cl} values is the line of identity $\alpha_{Cl} = 0$

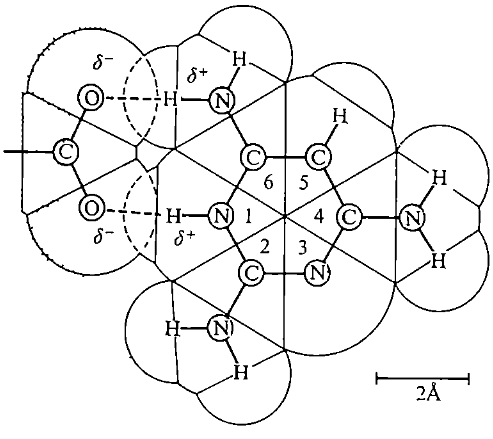

Fig 2 Scale drawing of a molecule of TAP associated by two hydrogen bonds to a carboxylic group The outline gives the van der Waal's surface, the heavy lines inside represent the internal covalent bonds, the broken lines represent hydrogen bonds The van der Waal atomic radii, bond lengths and angles are taken from Corey-Pauling-Koltun models Notice the good fit of the two hydrogen bonds If a $-PO_4H^-$ group is used instead of a $-COO^-$ group, the fit of the hydrogen bonds is equally good The shadow limit represents a ring of radius 6 Å, an equivalent radius attributed to bullfrog gallbladder tight junction cation channels[2,4]

The gallbladder actively transports an isotonic solution from the mucosal side The rate of this fluid transport measures (and is caused by) active transport of Na^+ from the mucosal to the serosal solutions[3] In the absence of $NaHCO_3$ in the solutions the rate (average ± s e) of the fluid transport in six frog gallbladders was 3 9 $\pm$ 0 6 μl h^{-1} cm^{-2}, and after addition of 10 mM TAP, 3 8 $\pm$ 0 8 μl h^{-1} cm^{-2} When 7 mM $NaHCO_3$ (which stimulates active transport[3]) was included in the bathing solutions, the rate of transport increased to 9 8 $\pm$ 1 5 (n = 3) μl h^{-1} cm^{-2} in the control, and to 9 9 $\pm$ 1 9 (n = 3) μl h^{-1} cm^{-2} in the presence of 10 mM TAP Therefore TAP at a concentration which blocks 80% of the transepithelial Na^+ conductance does not seem to interfere with active Na^- transport in frog gallbladder nor affect HCO_3^- stimulation of transport

A similar conclusion can be reached from a completely different observation in 24 frog gallbladders TAP (5–11 mM) increased R_m and the spontaneous transepithelial p d (V_m) from 129 $\pm$ 7 Ω cm^2 and 2 2 $\pm$ 0 2 mV (serosa positive), to 257 $\pm$ 12 Ω cm^2 and 4 8 $\pm$ 0 4 mV respectively But the ratio V_m/R_m (analogous to an open circuit current and hence a measure of active Na^+ transport) did not change significantly (17 1 $\pm$ 1 4 μA cm^{-2} for the control, against 18 3 $\pm$ 1 3 μA cm^{-2} after TAP) These results add to the growing evidence in support of an electrogenic Na^+ transport rather than the original suggestion[5] of an electrically neutral mechanism as responsible for active Na^+ transport in gallbladder (see ref 4 for further discussion)

Therefore, I conclude that in frog gallbladder TAP acts specifically in the cation-selective ion channels of the tight junction responsible for the passive Na^+ permeability[1,4] without affecting either the small Cl^- leakage current or the active Na^+ transport

From studies on the cation permeability and selectivity in frog gallbladder[2,4] these channels are thought to be hydrated and relatively large (the estimated radius is 6–8 Å) Their Na^+ conductance is controlled by acidic groups whose apparent pK_a is 4 4 The cation ligands in the channel (probably the same acidic groups) behave as strong hydrogen bond proton acceptor sites which distinguish more strongly among cationic proton donors than does water[6]

The protonation of TAP occurs at one of the ring nitrogen atoms (see Fig 2), but the net charge is delocalised by resonance, and shared with the 2,4 and 6 amino groups[7] Therefore, TAP should be a strong H bond proton donor The H bond association illustrated in Fig 2 between a molecule of TAP with a $-COO^-$ group (or a $-PO_4H^-$ group, although it is not shown in the figure) could be particularly strong, because the atoms involved in the H bonds have high partial ionic character and form appropriate angles between the internuclear lines (Fig 2) Actually, ion pair formation between phosphate ions and 2,4-diaminopyrimidine in relatively dilute aqueous solutions has been reported and attributed to H bond associations such as those illustrated in Fig 2 (ref 7) Indeed, these characteristics of TAP as a strongly H bonding proton donor were what led me to try it as a blocker of the strongly H bonding acceptor sites in gallbladder channels

Probably the value of K_m (equation 1) extracted from Fig 1 reflects the affinity constant of the binding of TAP with the ligands of the channel The ligands presumably bind TAP so strongly (compared with their binding to Na^+) that TAP is relatively immobilised and blocks the ligands and channel for Na^+ This affinity could result from an H bonded association of TAP with the strongly proton acceptor acidic groups of the channel, as illustrated in Fig 2

Figure 2 is only an attempt to explain the relatively high affinity of TAP to the acidic ligands of the channel, but other bonds and forces could be important By studying this interaction in detail, more information on the molecular structure of the channel is expected to be elucidated The tight junction cationic conductance accounts for most of the passive ionic transepithelial permeability in gallbladder (and probably most leaky epithelia) Because of its specificity and effectiveness in blocking this cation conductance, TAP constitutes a potential tool for the study of epithelial physiology and provides a pharmacological way of specifically controlling the ionic permeability across leaky epithelia

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Platelet macroglycopeptide

EVIDENCE from electron microscopy[1,2], chemical analyses of purified membranes[3,4] and the use of specific membrane labelling procedures[5], has demonstrated the presence of a carbohydrate-rich layer on the surface of the blood platelet This layer contributes many of the groupings[6] which give rise to the net electronegative charge on the outer surface of the plasma membrane There is evidence to suggest that the surface-bound carbohydrate is unusual in the platelet both in terms of the large size of the constituent glycoprotein units[7] and in the relatively high density of the terminal acidic monosaccharide, sialic acid[8]

Within the functions of the platelet as a haemostatic cell, various species differences have been shown to exist in terms of the response of the platelet to various stimuli such as ADP, 5-HT and adrenaline[9] But no differences have yet been shown in the structure of the plasma membrane, elements of which contain the groupings with which external stimuli first come into contact Here I have investigated the nature of the surface glycoprotein by examining the timed release of acidic glycopeptides from washed platelets by trypsin, followed by their characterisation on polyacrylamide gels Washed platelets obtained from human, patas monkey (*Erythrocebus patas*) and rabbit blood have been examined in this way A comparison of the glycopeptides released is shown in Fig 1

Trypsin digestion resulted in the rapid release of acidic glycopeptide, as located by Alcian Blue staining, in each of the three species examined With a digestion time of 1 min, one major band could be seen with only traces of others After 30 min digestion, however, a more complex pattern was apparent Two (human), four (rabbit) and six (patas monkey) bands were present, each band representing a glycopeptide, or class of glycopeptides of differing size or charge

Electrophoresis was performed employing standard 7% acrylamide separating gels[10] Staining capacity was adjudged to be due to complex formation between the Alcian Blue and terminal sialic acid moieties of the constituent oligosaccharide chains of the glycopeptides The staining capacity was lost following removal of the sialic acid by previous incubation with neuraminidase It was noticed that after 30 min digestion, the intensity of the first released glycopeptide was much reduced To ascertain whether the new bands were formed by further digestion of the platelet surface or by hydrolysis of the first glycopeptide, 1 min supernatants from rabbit platelet digests were concentrated, fractionated on Sephadex G-200, and the eluted fractions containing the glycopeptide again subjected to digestion with trypsin for 30 min in conditions identical to those of the original digestion of the washed platelets After 30 min, a similar glycopeptide pattern was obtained to that shown in Fig 1 Thus, it would seem that the later bands are produced as a result of hydrolysis of a single glycopeptide precursor. The major bands as now seen for each species appeared to resist further digestion by trypsin and were still present even after 18 h digestion

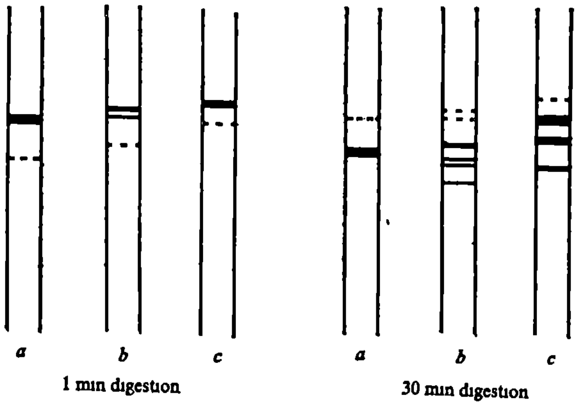

Fig. 1 Washed platelets (2 × 10^{9} ml^{-1}) suspended in 0 01 M Tris-HCl, *p*H 7 4, containing 0 15 M NaCl, 3 mM EDTA and 0 35% bovine albumin, were incubated in the presence of trypsin (0 2 mg ml^{-1}) at 37° C At selected time intervals aliquots were removed and added to soybean trypsin inhibitor (0 4 mg ml^{-1}) The platelets were sedimented by centrifugation at 1,500*g* at 4° C and 0 1 ml aliquots of the supernatant were transferred to 7% polyacrylamide separating gels (0 5 × 7 5 cm) in an even suspension of Sephadex G-25 powder A small aliquot of bromophenol blue was added as an internal reference standard Electrophoresis was performed at 200 V (constant voltage) for 1 25 h The gels were stained overnight in 1% Alcian Blue in 2% acetic acid, destained electrophoretically, and stored in 7% acetic acid at 4° C *a*, Human, *b*, patas monkey, *c*, rabbit,

Aliquots of the 1 min supernatants were also subjected to electrophoresis in the presence of the detergent sodium dodecyl sulphate (SDS) using an adaptation of the method of Weber and Osborn[11] One major glycopeptide band was seen for each species, the approximate molecular weight of which varied between 168,000 for the rabbit, 147,000 for the human and 142,000 for the patas monkey (Fig 2)

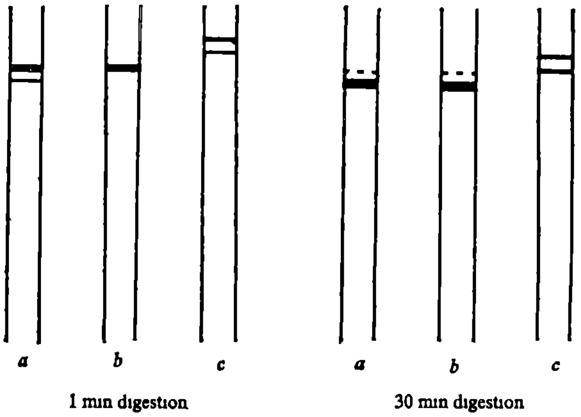

Fig. 2 Aliquots of the trypsin digest supernatants were incubated for 1 h at 37° C in the presence of 1% SDS and 1% 2-mercaptoethanol Following overnight dialysis against 0 01 M phosphate buffer, *p*H 7 1, containing 0 1% SDS and 0 1% 2-mercaptoethanol, glycerol and bromophenol blue were added to give final concentrations of 10% (w/v) and 0 02% (w/v) respectively Aliquots of 0 1 ml were applied to 7% (w/v) polyacrylamide gels containing 0 1% SDS and electrophoresis performed as described by Weber and Osborn[11] Glycopeptide was located by the periodate Schiff reaction[12] and molecular weight determinations made in comparison with the relative migration of a series of protein standards[11] *a*, Human, *b*, patas monkey, *c*, rabbit

After 30 min digestion, there was still one glycopeptide band in the human and patas monkey samples, however the approximate molecular weight of this band had fallen to 124,000 and 126,000 respectively In the rabbit two bands of molecular weight 144,000 and 159,000 were present In view of possible interference from sulphate groups contributed by the detergent, glycopeptide was here located by the periodate Schiff reaction[12] Substitution of this staining procedure for Alcian Blue in the characterisation of the glycopeptides of the 1 and 30 min digests as illustrated in Fig 1 showed no alteration in the pattern obtained, indicating a probable absence of large neutral glycopeptides

No difference in any of the electrophoretic patterns described was seen during examination of a number of individuals of each species Thus, it would seem that the glycopeptide patterns obtained were characteristic of the species examined The initial release of a large glycopeptide was common to each, though there were variations in the size of this molecule Later degradation of the parent glycopeptide by trypsin revealed differences in the number of glycopeptides obtained This is indicative of differences in the composition of the parent molecule, both in terms of its size and the number of acidic moieties carried So some differences in the molecular composition of the surface glycoprotein components of platelets of different species are apparent, though the possession of a large parent glycopeptide is common to each of the species examined here Further investigation of the role of the surface glycoproteins is suggested to determine their role in the reception of external stimuli and in governing the permeability of molecules to inner regions of the membrane

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Amplification of a circular segment of SV40 DNA

DURING serial undiluted passage of simian virus 40 (SV40) in monkey kidney cells, the viral genome undergoes genetic rearrangements involving the incorporation of cellular DNA sequences[1–5] as well as deletions and reiterations of specific viral DNA sequences[3,6–8] In previous work[7], a mutant of an SV40 variant (DAR, refs 9–11) was identified after serial passage of DAR virus in primary green monkey kidney cells This mutant DNA was physically mapped by hybridising it to specific DNA segments produced by restriction endonuclease cleavage of SV40 DNA[8] The mutant DNA was found to contain only one-third of the SV40 genome which was reiterated three times to produce a DNA molecule the size of wild-type SV40 ($3\ 2\times10^{6}$ daltons) Most of the early gene sequences and a portion of the late gene region[12] were deleted from the triplicated segment, but it retained the normal site for initiation of viral DNA replication[8]

The restriction endonuclease from *Escherichia coli*, R *Eco*RI, introduces one unique cleavage in SV40[13,14] or DAR[15] DNA to yield unit length linear molecules with short cohesive termini[16] In the DAR triplication mutant, the R *Eco*RI site is symmetrically triplicated, therefore, after R *Eco*RI cleavage, segments are formed which are one-third the size of SV40 (DAR) DNA[7]

In this study, the one-third segments (10 4S DNA) arising after R *Eco*RI cleavage of DAR DNA have been isolated These segments, which contain cohesive termini, have been purified, converted to hydrogen bonded circles and covalently closed by phage T4 polynucleotide ligase to produce circular DNA segments ('minicircles') We have used the purified 'minicircular' molecules to infect monkey kidney cells in the presence of helper SV40 DNA molecules

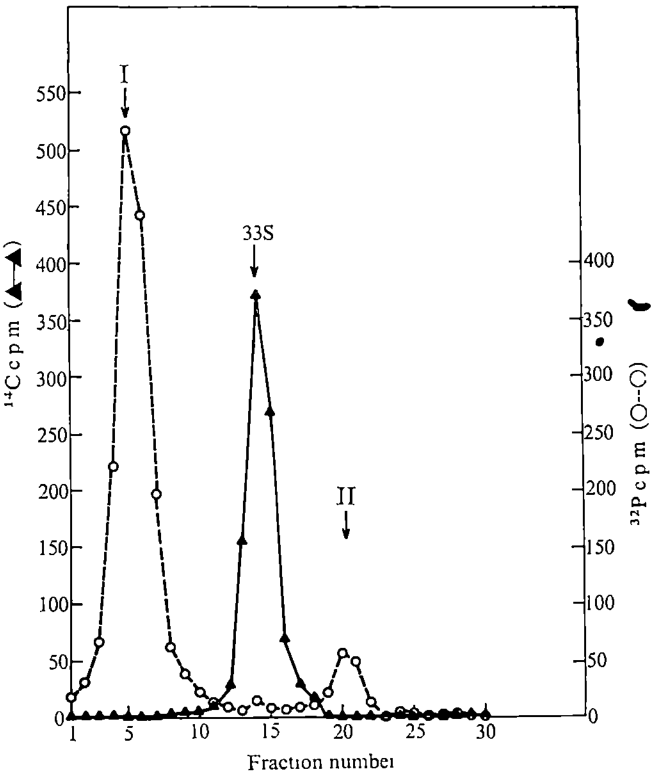

Fig 1 Analysis of minicircular DNA by alkaline sucrose sedimentation The minicircular DNA, formed by ligase treatment of 10 4S segments, was mixed with a sample of ^{32}P-labelled SV40 DNA I (53S) and II (16S) and then sedimented through a 5–20% alkaline sucrose gradient for 130 min at 50,000 r p m in the SW 50 1 rotor[19] DAR ^{14}C DNA I (ref 17) was cleaved in a reaction mixture (0 5 ml) consisting of 67 mM Tris-HCl, (*p*H 7 6), 10 mM $MgCl_2$, 15 μg of DAR viral DNA, and 0 015 ml of R *Eco*RI[7] (10^4 units ml^{-1}) After incubation at 37° C for 30 min, the reaction was stopped by chilling to 0° C and adding 10 μmol of EDTA (*p*H 8 0) The 10 4S DNA segments were isolated by sedimentation through a 5–30% neutral sucrose gradient using the Spinco SW41 type rotor[7] The pooled fractions were extracted three times with redistilled phenol and dialysed extensively against 0 01 M Tris-HCl (*p*H 7 5), 0 01 M NaCl, and 0 001 M EDTA at 4° C The ligase reaction mixture (5 ml) consisted of 66 mM Tris-HCl (*p*H 7 6), 7 mM $MgCl_2$, 70 μM ATP, 5 μg of 10 4S DNA and 1 5 units of phage T4 ligase[17] After 16 h at 18° C, additional ATP (0 35 μmol) and ligase (1 5 units) were added and the incubation was continued for 5 additional hours Then EDTA was added to a final concentration of 14 mM and the minicircular DNA was purified in an ethidium bromide-CsCl density gradient containing 200 μg ml^{-1} of ethidium bromide[19]

Mertz and Davis[16] have demonstrated that DNA molecules cleaved by endonuclease R *Eco*RI can be converted to closed circles *in vitro* because short cohesive termini are created by the staggered nucleolytic cleavage A DAR^{14}C-DNA I preparation, containing 80% triplicated DNA, was cleaved with endonuclease R *Eco*RI and the 10 4S DNA segments were purified by neutral sucrose rate zonal centrifugation[7] Minicircles were then formed from the 10 4S linear segments using bacteriophage T4 polynucleotide ligase[17], at a final DNA concentration of 1 μg ml^{-1} on order to prevent the formation of concatamers and circular oligomers[16] Approximately 80% of the 10 4S segments were covalently closed in the ligase reaction After the prolonged incubation at 18° C, the covalently closed, circular DNA was purified by dye-density gradient centrifugation A portion of the heavy band material was then analysed by alkaline sucrose sedimentation (Fig 1) The minicircles of DAR (SV40) DNA have a sedimentation value of 33S as compared to 53S for superhelical SV40 DNA I molecules No other DNA species such as dimeric or other oligomeric forms of the 10 4S segments were detectable

When the CV-1 line of monkey kidney cells[18] was infected with the purified minicircles in the absence of SV40 DNA I, there was no detectable incorporation of ^{3}H-thymidine

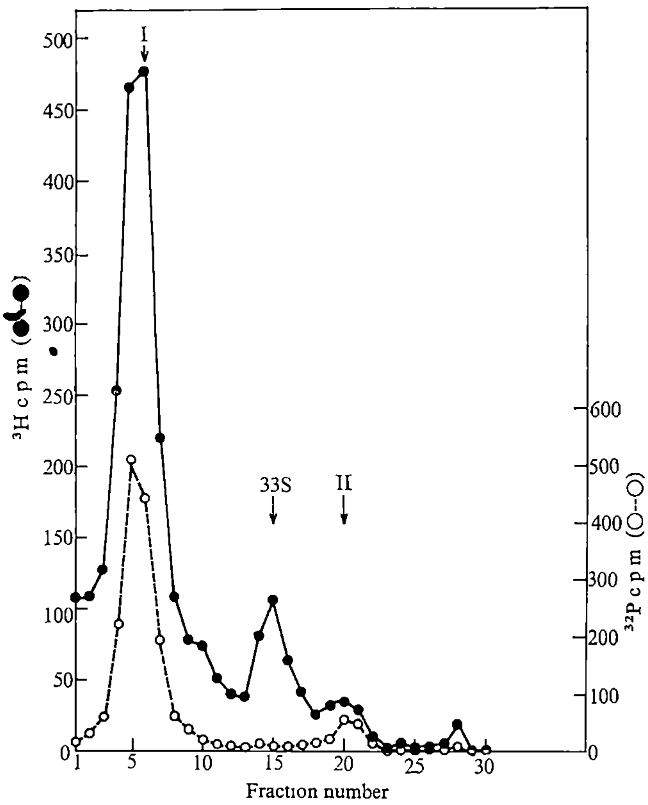

Fig 2 Alkaline sucrose sedimentation analysis of intracellular DNA I from CV-1 cells infected with SV40 and minicircular DNA All DNA infections were performed according to the method of McCutheon and Pagano[20] Thus for infection with minicircular ^{14}C-DNA, or infection with SV40 DNA I and the minicircular DNA together, confluent monolayer cultures of CV-1 cells[18] in 75 cm^2 plastic flasks (Falcon) were rinsed twice with 20 ml portions of warm PBS (0 008 M Na_2HPO_4, 0 0015 M KH_2PO_4, 0 14 M NaCl, and 0 003 M KCl) The DNA sample (0 8 ml) was then added to 0 4 ml of DEAE-dextran (2 mg ml^{-1} in PBS) to give a final concentration of 2 μg ml^{-1} for SV40 DNA, and 0 5 to 1 0 μg ml^{-1} for the minicircular DNA For infection with SV40 DNA and the minicircular DNA together, the final concentration of each DNA was the same as in the individual infections At 60–70 h after DNA infection of CV-1 cells, the medium (Eagle's MEM) was removed and replaced with medium containing 100 μCi ml^{-1} of ^{3}H-thymidine (6 ml per flask, 50 Ci mmol^{-1}) After 2 h at 37° C, the viral DNA was extracted by the method of Hirt[21] and the supernatant solution was dialysed against 0 01 M Tris-HCl, (pH 7 5), 0 01 M NaCl, 0 001 M EDTA at 4° C Superhelical viral DNA was purified by two cycles of equilibrium centrifugation in CsCl–ethidium bromide density gradients and analysed as described in the legend to Fig 1

into superhelical molecules banding with SV40 DNA I in dye-density gradients or sedimenting at 33S in alkaline sucrose This was not unexpected since the 10 4S DNA segments lack most of the early region for SV40 gene expression[8] In contrast, when cells were infected with approximately equivalent numbers of minicircular and SV40 DNA molecules, ^{3}H-thymidine was incorporated into superhelical molecules having the same density as SV40 DNA I in dye-density gradients (data not shown) When the purified, ^{3}H-labelled, superhelical DNA was analysed by alkaline sucrose sedimentation (Fig 2), a major peak at 53S and a minor peak at 33S were observed These results suggest that replication of the minicircular segments requires one or more gene product supplied by the helper genomes

The composition of the superhelical, ^{3}H-labelled DNA from the mixed infection was further analysed by neutral sucrose rate zonal centrifugation In Fig 3*a*, it is evident that, in addition to molecules sedimenting at 21S (viral genomes of normal size) and at 13 5S (superhelical minicircles), a substantial fraction (26%) of the DNA sedimented more rapidly than SV40 DNA I A portion of this heterogenous, superhelical DNA preparation analysed in Fig 3*a*, was digested with endonuclease R*Eco*RI and analysed by neutral sucrose sedimentation (Fig 3*b*) Two major bands with peaks at 14 5S (SV40 DNA III) and at 10 4S (one-third segments) were identified The proportion of molecules (37%) yielding 10 4S segments after cleavage was unexpectedly large considering that the 13 5S band in Fig 3*a* comprised only 7% of the labelled DNA This suggested that part of the 10 4S DNA was present in DNA molecules which were larger than one third of the size of SV40 DNA

In order to determine whether 10 4S DNA segments had been triplicated or more extensively amplified, different regions of a preparative neutral sucrose gradient were pooled as indicated in Fig 3*a* on the horizontal, solid line (pools A–D)

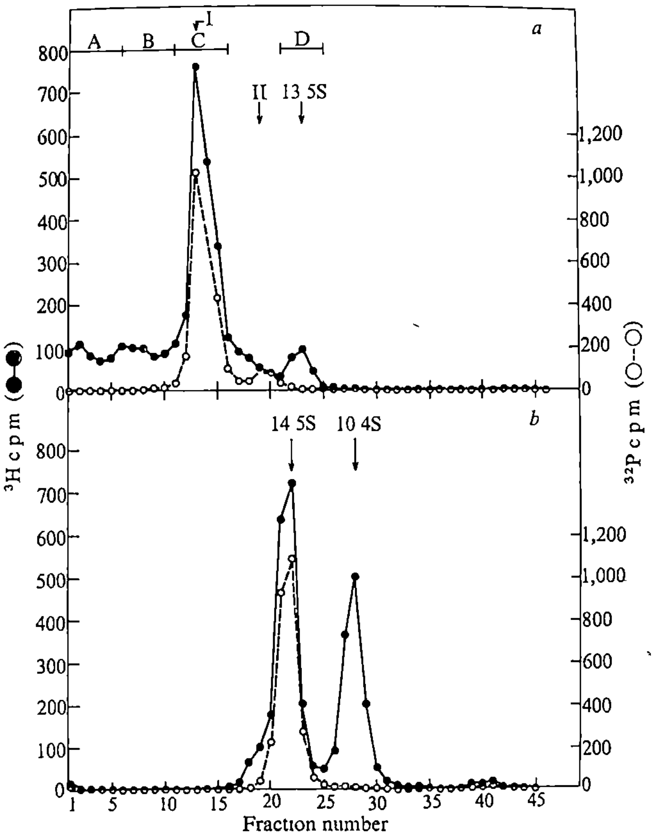

Fig. 3 R*Eco*RI cleavage of intracellular DNA I from CV 1 cells infected with SV40 and minicircular DNA Superhelical viral DNA was isolated as described in the legend to Fig 2 and analysed[19] by neutral sucrose gradient centrifugation (7 h, 40,000 r p m) in the Spinco SW41 rotor before (*a*) and after (*b*) cleavage with R*Eco*RI

Each of these DNA preparations was analysed separately before and after R*Eco*RI cleavage by neutral sucrose sedimentation (Fig 4) Fractions from pool A (Fig 4*a*) comprised 12% of the total superhelical DNA and had an average sedimentation value of 27 6S (molecular weight= 5 7×10^6, ref 22) After R*Eco*RI cleavage (Fig 4*d*), a peak at 10 4S (51% of pool A) was present with two less well defined peaks at 14 5S and at 16 7S Pool B (Fig 4*b*) had an average sedimentation value of 25 3S (molecular weight =4 7×10^6) and, after cleavage (Fig 4*e*), produced molecules which sedimented at 14 5S (25%) and at 10 4S (64%) After cleavage of pool C, which sedimented with SV40 DNA I (21S), 78% of this DNA sedimented at 14 5S while 18% had a sedimentation value of 10 4S Pool D (data not shown) produced only 10 4S DNA after cleavage, as ex-

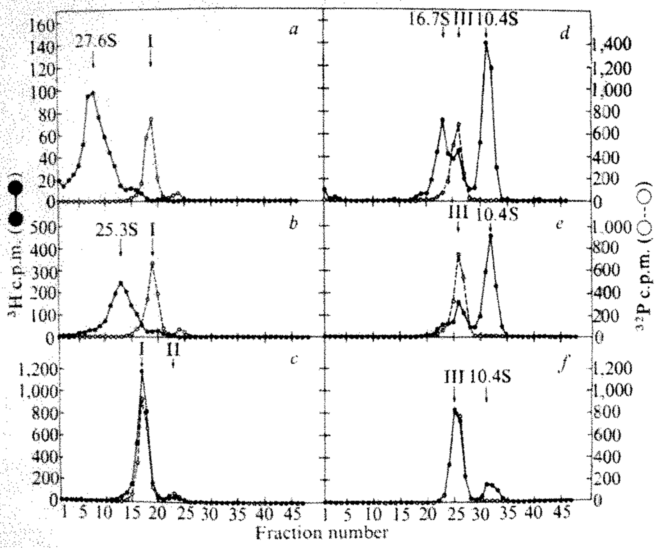

Fig. 4 Analysis of partially fractionated oligomers isolated from cells infected with SV40 and minicircular DNA. Different regions of a preparative neutral sucrose gradient similar to Fig. 3*a* were pooled as indicated in Fig. 4*a* on the horizontal solid line. Each of these DNA preparations was then analysed separately before and after cleavage by R·*Eco*RI as described in the legend to Fig. 3. The analyses of pool A are shown in *a* (before cleavage) and *d* (after cleavage), pool B in *b* and *e* and pool C in *c* and *f*.

pected. These results show that molecules of much higher molecular weight than the minicircular DNA are a source of 10.4S DNA after R·*Eco*RI cleavage and suggest that amplification of the 10.4S segments had occurred producing circular oligomers of this segment.

When the mixed infection of CV-1 cells with minicircular and SV40 DNA was allowed to proceed to completion, the virions purified from the lysate formed only one band in a CsCl density gradient[11]. DNA extracted from the purified virions sedimented in a narrow band in a neutral sucrose gradient at the position of SV40 DNA I (data not shown). Thus, only molecules having the same size and shape as SV40 DNA were encapsidated. After R·*Eco*RI cleavage of the DNA from virions, molecules sedimenting at 14.5S and 10.4S were found, indicating that trimeric genomes had been encapsidated. Furthermore, cleavage of these 10.4S segments with the restriction endonuclease from *Hemophilus influenzae* produced five fragments having the same electrophoretic mobility as those produced from the original 10.4S DNA[8].

These results indicate that circular, duplex viral DNA segments much smaller than SV40 DNA can be replicated *in vivo*. In addition they support the model proposed by Khoury *et al.*[8] for the generation of the original DAR triplication mutant. In our experiments, cells were infected with a minicircular DNA molecule, formed *in vitro*, which then served as a precursor *in vivo* in the formation of trimers and higher oligomers as predicated by the proposed model. The DAR triplication mutant first appeared after the third passage in primary monkey kidney cells and rapidly became the predominant species in later passages[7]. This mutant may have had a strong selective advantage since it contained three sites for initiation of DNA synthesis. The oligomeric forms in our studies may have evolved for a similar reason.

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Leukaemogenic effect of repeated inoculations with small doses of Tennant virus in BALB/c mice

DOSE-RESPONSE studies of oncogenic viruses in animals are usually restricted to single inoculations. The study of the effect of dose fractionation has been a rather neglected subject in this field. An important question is whether the combined effect of the small doses is less than the effect of a single exposure to the same total dose. In this study on dose fractionation of a murine leukaemia virus we observed the opposite effect.

The B/Tennant leukaemia virus was isolated from a spontaneous lymphosarcoma of BALB/c mice[1]. It usually induces this disease in BALB/c mice with a relatively short latency period[1–3]. Dr J. Tennant (Sloan–Kettering Institute, New York) sent us a sample of the virus in 1969 and we then passed it continuously by inoculations of crude cell-free extracts of leukaemic tissues into neonate BALB/c mice. For the present study, 1 l of a 10% cell-free extract was prepared as previously described[4] and stored in liquid nitrogen in 10 ml aliquots. Intraperitoneal inoculations with virus were started in female BALB/c mice (20 per group) when they were six weeks old.

Table 1 Leukaemia incidence and latency in BALB/c mice repeatedly injected with Tennant murine leukaemia virus

Virus dilution	Number of inoculations 1	3	6	12	As many as possible till autopsy
10^{-1}	100 (44±8)*	100 (43±5)	100 (38±10)	100 (41±8)	100 (35±4)
10^{-2}	100 (49±5)	100 (49±6)	100 (47±6)	100 (43±5)	100 (47±7)
10^{-3}	100 (80±18)	100 (67±22)	100 (59±11)	100 (58±15)	100 (49±6)
10^{-4}	35 (109±3)	20 (82±14)	75 (79±19)	100 (64±20)	100 (56±13)
10^{-5}	0	0	25 (121±18)	65 (74±14)	100 (69±20)
10^{-6}	0	0	0	80 (99±8)	100 (72±11)

* %Incidence (latency period (d) ± s.d.).
† The average number of injections in these series is 15, 19, 20, 22, 28 and 29 respectively.

The mice were injected three times a week. They were examined twice a week for splenomegaly and/or swollen lymph nodes. The animals were then autopsied and histologically examined for confirmation of the development of leukaemia. The survivors were kept under observation only up to the age of 10 months. After that age, the first spontaneous lymphoreticular neoplasms begin to appear in this strain.

From the results in Table 1, it is obvious that a 1,000-fold dilution is still fully leukaemogenic. Higher virus doses or repeated inoculations tend to shorten the latency period. There is no indication that dose fractionation leads to a lower incidence or longer latency.

In fact we found that repeated inoculations with low doses of virus produce a significantly higher leukaemia incidence than a single injection with a much higher dose (for instance, 12×10^{-6} instead of 1×10^{-4}). A possible explanation is that continuous exposure to even very small numbers of virus particles induces immunological unresponsiveness to the virus, so that these small numbers may be propagated in the host. Alternatively, autointerference induced by a moderate dose of virus may play an important part.

It would be worthwhile to investigate whether a similar procedure can overcome the genetic resistance of some mouse strains to the virus[2,5], as well as that of adult rats. The observation must be extended to other tumour virus systems. If it proves to be of a general nature, it has important biohazard implications for tumour virological workers or investigators handling cell cultures which produce small amounts of C-type oncornavirus particles[6].

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New Giemsa method for the differential staining of sister chromatids

If human lymphocytes[1] or Chinese hamster[2] cells are treated with the base analogue 5-bromodeoxyuridine (BrdU) in the latter part of the S period, Giemsa stained chromosomes exhibit a pattern of condensed and extended segments along their length. This phenomenon has been attributed to a delay in the spiralisation pattern of the late replicating regions along the chromosomes. Other experiments[3] with Chinese hamster ovary (CHO) cells have shown that after two rounds of replication in the presence of BrdU or IdU, sister chromatids stain differentially with Giemsa, allowing the identification of the two chromatids, and the observation of sister chromatid exchanges (SCEs) without recourse to autoradiography. The chromatid with the bifilarly substituted DNA (BrdU substituted in both strands of DNA) is less condensed and stains more weakly with Giemsa than the unifilarly substituted sister chromatid. The yield of SCEs is approximately that observed by autoradiography.

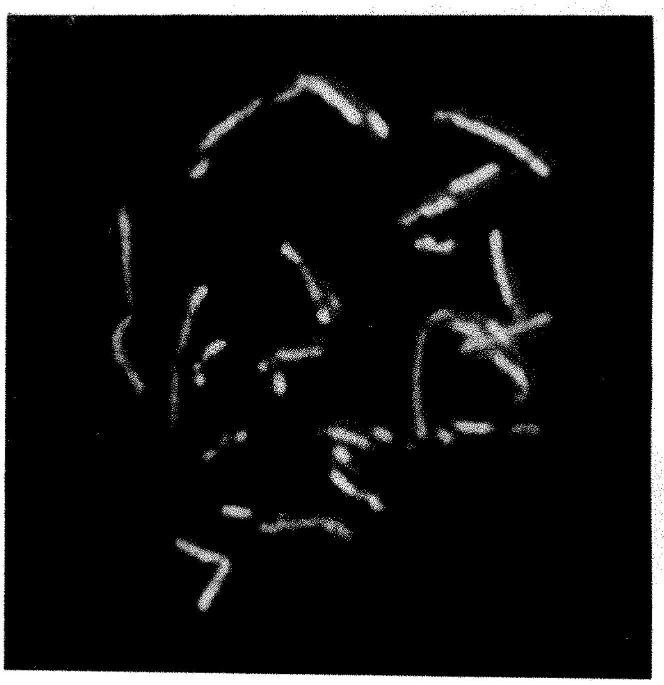

Fig. 1 CHO cell stained with Hoechst 33258 after two rounds of replication in the presence of BrdU. Fluorescence performed as in ref. 4. Leitz ortholux microscope, 200 W mercury light source UG-1 filter and TK400 dichroic mirror for excitation, and K400 and K430 barrier filters.

Latt[4] has described a fluorescence technique for human chromosomes using the dye Hoechst 33258. This dye normally fluoresces intensely when bound to poly(dA-dT), but when bound to poly(dA-BrdU) the fluorescence of the dye is quenched. After two rounds of replication in the presence of BrdU, followed by staining in Hoechst 33258, the unifilarly substituted chromatids fluoresced more brightly than the bifilarly substituted sister chromatids. Sister chromatid exchanges were readily observed. Though this technique provided a degree of resolution for the detection of SCEs which was previously not possible, the dye demonstrated a remarkable degree of photoinstability, so that the image faded very rapidly. This paper describes a technique in which the use of Hoechst 33258 is combined with Giemsa staining (fluorescent plus

Giemsa or FPG) to make permanent Giemsa-stained cytological preparations of differentially labelled chromatids.

Chinese hamster ovary (CHO) cells were cultured in McCoy's 5A medium supplemented with 13% foetal calf serum. Exponentially growing cells were treated with BrdU (final concentration 10 μm) for 24 h during which time two rounds of replication occurred.

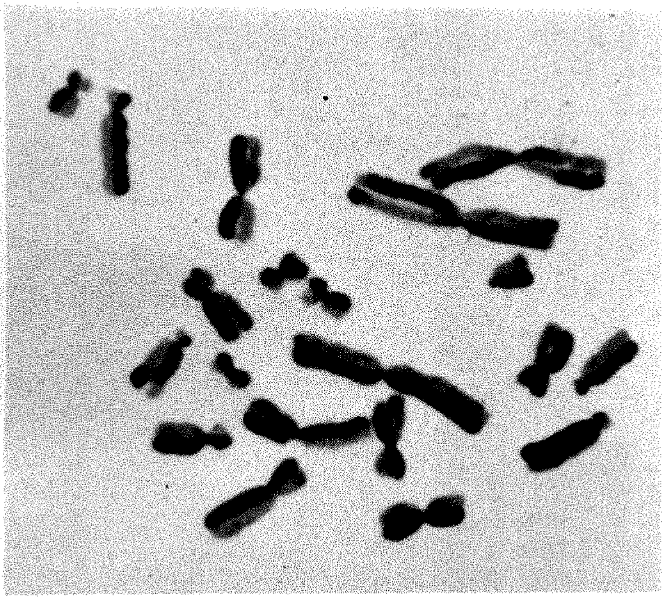

Fig. 2 Cell stained with Hoechst 33258 and then with Giemsa.

All cultures containing BrdU were kept in the dark to minimise the numbers of sister chromatid exchanges caused by the photolysis of BrdU containing DNA[3]. Colcemid (final concentration 2×10^{-7} M) was then added for 2 h and the mitotic cells collected by shaking. The cells were treated for 8 min with 0.075 M KCl to spread the chromosomes and then fixed in methanol acetic acid (3:1).

Drops of cells in fixative were placed on clean microslides and allowed to dry. The slides were then stained in Hoechst 33258 (0.5 μg ml^{-1} deionised water) for 12 min and rinsed briefly in deionised water. The preparations were mounted in water and the cover slip ringed with Holdtite rubber cement to prevent evaporation. Sister chromatids now fluoresced differentially (Fig. 1) as Latt has reported[1].

These same preparations were then allowed to age for 24 h, after which time the coverslips were removed and the slides incubated for 2 h at 60° C in either 2×SSC (0.3 M sodium chloride–0.03 M trisodium citrate) or in water. This was then followed by 30 min staining in 3% Giemsa solution (Gurr's R66 *p*H 6.8). If SSC was used G bands could sometimes be seen.

Figure 2 shows a cell after Hoechst 33258 and Giemsa staining. The Giemsa preparation is now permanent, that is does not fade rapidly, and can be studied at leisure with the compound microscope. The chromatid that incorporated BrdU into both strands of the DNA helix fluoresced dimly and now stains weakly with Giemsa whereas the chromatid that incorporated BrdU into only one strand fluoresced brightly and now stains darkly with Giemsa.

Latt[1] has suggested that the difference between the two chromatids is caused by a differential quenching of fluorescence by the BrdU incorporated in either one or two strands of the DNA. Ikushima and Wolff[3], however, have postulated that differences in the binding of protein to BrdU-containing chromatids is the cause of the differential staining with Giemsa. The differential Giemsa stainability observed here of chromatids pretreated with Hoechst 33258 indicates that the difference cannot be attributed simply to quenching of fluorescence.

Table 1 Sister chromatid exchanges (SCE) observed in CHO cells after two rounds of replication in the presence of BrdU

Stain	(No. of SCE)/ (No. of chromosomes)	No. of SCE per chromosome
Hoechst 33258	360/554	0.65±0.034
Hoechst 33258 followed by Giemsa	149/223	0.668±0.055

This differential staining of the two sister chromatids can also be observed if the cells are stained for 5 min with 0.005% acridine orange in Sörensen's buffer at *p*H 6.7, but is only barely seen, if stained with quinacrine. The difference in fluorescence between the two chromatids, however, is not readily distinguished until the cells are exposed to exciting light passed through a BG 12 filter. Only those cells so exposed show a subsequent differential staining of sister chromatids with Giemsa. If, however, too much exciting light falls on the cell and too much fading of the fluorescence occurs, then the subsequent Giemsa staining is very weak. Similarly, if cells stained with Hoechst 33258, which fades very rapidly, are exposed to exciting light they ordinarily do not stain as well with Giemsa as unexposed cells.

The number of SCEs observed when cells are stained with Hoechst 33258, with or without subsequent Giemsa staining, is greater than the number observed after staining in Giemsa alone[3] or after autoradiography[5]. In addition all chromosomes in a cell can be scored whereas after autoradiography only the larger chromosomes are scorable.

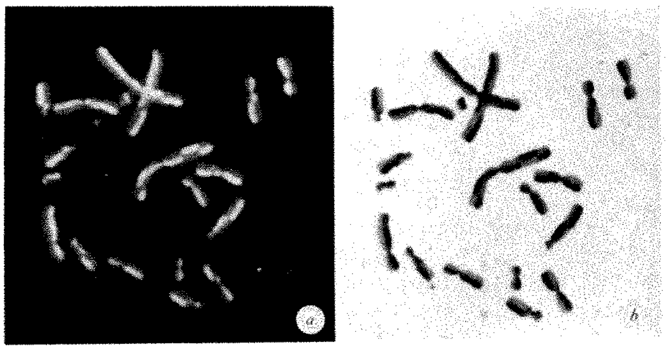

Fig. 3 Cell stained first with Hoechst 33258 (*a*) and subsequently with Giemsa (*b*).

Table 1 presents results of an experiment in which cells were grown in 5 μM BrdU. The frequency of sister chromatid exchanges observed in 33258 plus Giemsa-stained cells was the same as that observed in cells stained with 33258 alone. Furthermore, in Fig. 3 it may be seen that the same exchanges are observable by each technique. The FPG technique does seem to offer some additional resolution, however. We have noticed that when small double exchanges occur near the tips of chromosomes, these can be seen clearly with Giemsa, whereas after fluorescent stain alone, because of flare, both tips often appear to fluoresce and thus mimic isolabelling as seen in autoradiographic preparations.

It therefore seems that the technique presented here has all of the advantages of the fluorescent technique discovered by Latt, but has the additional advantages of producing nonfading permanently stained cells that do not require fluorescence microscopy and photography for their study.

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Effect of cysteine on the survival of mice with transplanted malignant thymoma

TISSUE collagenase has been detected in inflamed human tissues by Fullmer and Gibson[1], and in some malignant neoplasms by Riley and Peacock[2]. The possible role of tissue collagenase in neoplastic spread has been suggested by Birbeck and Wheatley[3] from an electron microscopic study of the invasion of ascites tumour cells into the abdominal wall of BALB/c mice. They reported that the tumour cells appeared to be actively destroying adjacent normal tissues, which is suggestive of the activity of tissue-destroying enzymes derived from the neoplastic cells. The finding of collagenase activity in human and animal tumours by Riley and Peacock[2], Campbell[4], and Dresden, Heilman and Schmidt[5], prompted a study into the effect that collagenase inhibitors might have on the spread of neoplastic disease. It has been reported by Brown and Weller[3] that corneal ulcerations which inevitably occur following severe alkali burns are due to endogenously produced collagenase and that they can be prevented by topical application of solutions of collagenase inhibitors such as cysteine. The present investigation was designed to test our hypothesis that collagenase produced in malignant neoplasms is a factor in neoplastic invasion and that by inhibiting its activity the arrest of a malignant neoplastic disease could be achieved. The results reported here of initial experiments demonstrate the beneficial effects of cysteine on the survival rates of animals into which malignant thymomas were transplanted.

The malignant thymoma was originally obtained from the Walter and Eliza Hall Institute, Melbourne, where it had been repeatedly transmitted in C57Bl mice. Fresh specimens of thymoma, which have been shown in our laboratories to produce collagenase on *in vitro* cultivation, were cut into 1 mm^3 and then dispersed into single cell suspensions by teasing in sterile 0.15 M NaCl.

Table 1 The effects of cysteine on the survival rates of animals after transplantation of malignant thymomas

Treatment	Mean survival time (d)	Weighted mean survival time (d)
Thymoma alone	31	31
Thymoma + 0.15 M NaCl	34	45
Thymoma + 0.01 M cysteine	37	37
Thymoma + 0.1 M cysteine	37	37
Thymoma + 0.25 M cysteine	65*	98
Thymoma + 0.5 M cysteine	58	86
Thymoma + 1.0 M cysteine	54	65

* Student's *t* test significant at the $P < 0.05$ level.

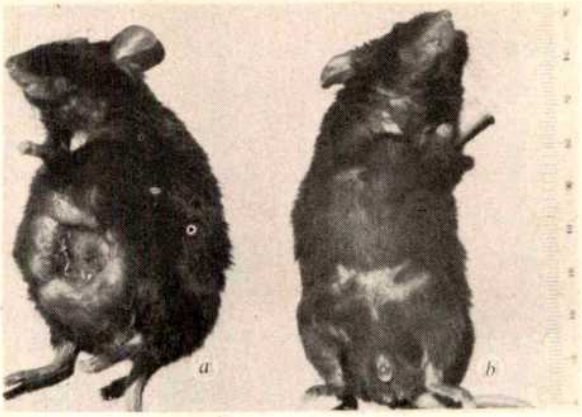

Fig. 1 *a*, C57Bl mouse injected with thymona cells alone died at 38 d. *b*, C57Bl mouse injected with thymoma cells +0.25 M cysteine and killed at 100 d.

Approximately 10^7 viable cells in 0.3 ml of 0.15 M NaCl were infused subcutaneously into the abdominal tissues of male C57Bl mice weighing 20–25 g. The study was designed so that there were seven groups, each with six animals, treated as shown in Table 1. For 8 d, 0.2 ml of the varying concentrations of cysteine; 0.01 M, 0.1 M, 0.25 M, 0.5 M and 1 M; and 0.15 M NaCl as a control, were infused as near as could be determined to the same site as the depot of thymoma cells, commencing 10 d after the initial inoculation of thymoma cells. The macroscopic difference between mice injected with thymoma cells and given no therapy, as illustrated in Fig. 1*a*, or given 0.2 ml of 0.25 M cysteine daily for eight days, as illustrated in Fig. 1*b*, is clearly evident.

The results of the experiment are illustrated in Table 1 and show that 0.25 M cysteine injections significantly ($P<0.05$) prolonged survival time as compared with the saline treated controls and to higher and lower doses of cysteine. The weighted mean (Table 1) takes into account the number of surviving animals at any particular point of time and was calculated according to the method described by Zippin and Armitage[8] using 100 d as the end point of the experiment and highlights in these experiments the difference between control and experimental animals, especially those in which 0.25 M cysteine was administered.

The results of this study suggest that 0.25 M cysteine, introduced locally, is the most effective concentration to inhibit the growth of transplanted malignant thymomas in C57Bl mice.

If the effect of the cysteine is due to its collagenase inhibiting effect described in the literature by Slansky and Gnadinger[9] and Brown and Weller[10], and if the limitations of tumour growth in the described experiments was due to this effect, an important area of investigation is suggested because of the known ability that some malignant neoplasms have of producing tissue collagenase.

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Occurrence of lead in tuna

AT a recent national conference in the United States on baseline studies of pollutants in the marine environment[1] serious doubts were raised about the validity of most published measurements of lead concentrations in seawater and in marine organisms Available data for foods and other plant and animal tissues are also implicated because they may contain similar concentrations of lead

It was recommended at the baseline meeting that a workshop be established to carry out interlaboratory analyses of lead in seawater The workshop, sponsored by the National Science Foundation Office for the International Decade of Ocean Exploration (IDOE), was initiated in the autumn of 1972 Samples of seawater standardised at one university by isotope dilution were circulated among participating oceanographic laboratories at seven universities in the United States and at one ministry in the United Kingdom None of the participants was aware of the standardised value until all results had been reported Results of the workshop confirmed suspicions that not one oceanographic laboratory of superior status could, as of September 1973, collect and analyse lead in coastal surface seawater by atomic absorption or anodic stripping voltammetry techniques and report reliable values[2,3] It seems that most published measurements of lead in seawater may be erroneously high by factors of 10 to 100

We have now determined lead concentrations in tuna fish by a reliable analytical technique The observed lead concentrations in muscle are four orders of magnitude lower than those in epidermis Both values lie so far away from generally accepted levels of lead in tissues that analysts may find it difficult to recognise that such lead concentrations actually exist These new findings strongly suggest, as in the case of seawater, that recent studies of the hazards of lead pollution may be misleading if they are based on analyses of plant and animal tissues determined by routine analytical methods carried out without the use of clean-laboratory techniques and without the necessary sensitivity and accuracy

The lead in tuna data are presented here as frames of reference for investigators applying atomic absorption or potentiometric analytical methods to studies of lead in plant and animal tissues

Our data were determined in two laboratories by stable isotope dilution using themal ionisation source high resolution mass spectrometers and special clean-laboratory chemical techniques[2,4] We analysed five types of samples (i) tuna muscle uncontaminated with lead by handling, and containing such small concentrations (~0 0003 p p m wet) that they must be determined with special care, (ii) tuna epidermis, where lead is found concentrated to levels (~2 p p m wet) that are poisonous to humans, (iii) tuna scales and dermis, which contain much less lead than epidermis, (iv) commercial canned tuna muscle, which is contaminated with industrial lead by a factor of 1,000 above levels in living tuna, and (v) a tuna muscle interlaboratory reference that could not be analysed properly by investigators using atomic absorption and anodic stripping techniques in oceanographic laboratories because it did not contain sufficient lead (~0 4 p p m dry), even though it had been contaminated with industrial lead by a factor of 400 above levels in living tuna during preparation by a commercial food manufacturer

Frozen tuna were obtained from IDOE The fish were thawed at 4° C for 24 h, and then exposed to room temperature for 1 h, leaving them still partially frozen and suitable for dissection Clean-room procedures were used to prevent industrial lead contamination, but additional precautions had to be taken to minimise transfer of mucosal slime containing large amounts of lead to interior tissues during dissection

Water was removed from tissues either by heating to 110° C or by vacuum desiccation Values for water contents are approximate because of dehydration and plasma losses between the times when the fish were caught and the thawed tissues were weighed Three methods for decomposing tissue for analysis were used in two laboratories muffle furnace oxidation of 15–40 g wet tissue samples was used at the Scripps Institution of Oceanography, and either low temperature ashing with an excited ozygen plasma or wet acid oxidation of 0 5–4 g wet tissue samples were used at the California Institute of Technology Weighed amounts of lead isotope tracer were homogenised with the lead in the samples during digestion and oxidation, the lead isotope mixtures were isolated by ion-exchange and solvent extraction, and were then analysed in a mass spectrometer The amounts of lead in the samples were calculated from the observed ratios of $^{207}\mathrm{Pb}_{\mathrm{sample}}$ to $^{208}\mathrm{Pb}_{\mathrm{tracer}}$ and the weights of tracer Concentrations were calculated from sample weights

Results for tuna analyses are listed in Table 1 Observed lead concentrations in tuna muscle were so small there was

Table 1 Lead in tuna

Sample Type	Tissue	p p m Pb (wet)	wt % H_2O
8 kg Yellowfin (*Neothunnus macropterus*)	Pectoral fin	5 3	42
8 kg Skipjack (*Katsuwanus pelamis*)	Pectoral fin	4 1	38
7 kg Albacore (*Thunnus alalunga*)	Pectoral fin	0 45	32
(oxidise in excited oxygen plasma)	Epidermis	1 7	67
	Epidermis	1 4	67
	Scales	0 06	25
	Dermis	0 01	44
(acid digest at atmospheric pressure)	Gill filaments	0 014	73
	Muscle	0 0002*	71
	Muscle	0 0003*	71
(acid digest at high pressure in bomb)	Muscle	0 0003*	71
Canned tuna (in oil) cannery A	Muscle	0 10	57
Canned tuna (in oil) cannery B	Muscle	0 93	57

* Uncertainty is ~30% for these values Concentrations are three times larger than the present limit of measurement for a 4 g sample, which is set by the total analytical blank At this time this blank is 1 8 ng Pb

Table 2 Lead in US NBS reference materials by three different decomposition methods

Reference material	Muffle furnace (SIO)	Excited oxygen plasma and wet acid (CIT)
Bovine liver (as received)	0.333 (20% spread)	0.328 (5% spread)
Tuna muscle (vacuum dried)	0.22 to 0.35	0.34 to 0.45

All figures are expressed in p.p.m., and are means from multiple analyses.
SIO, Scripps Institution of Oceanography. CIT, California Institute of Technology.

concern whether proper mixing of tracer and sample lead isotopes had been achieved. Results obtained by hot HNO_3–$HClO_4$ decomposition at atmospheric pressure were checked by carrying out parallel acid decompositions in Teflon bombs at 180° C and at very high pressures. No significant differences in lead concentrations were observed between the two methods, so we think that proper isotopic equilibration had been achieved at ordinary temperatures and pressures. It seems likely that fresh tuna muscle, uncontaminated by handling, contains about 0.0003 p.p.m. Pb. This contrasts with the very high concentrations of lead in epidermis, which are about 2 p.p.m. in wet tissue. Humans are poisoned if they ingest food containing several p.p.m. Pb for a prolonged period.

It is doubtful that this excessive epidermal lead is an artefact derived from fishing operations because, as shown in Table 1, we have found similar concentrations in whole pectoral fins from different tuna obtained under various circumstances. On a mass balance basis, the lead content of fish fins is determined almost entirely by the concentration of lead in epidermis. The mucin secreted by the mucous cells of the epidermis contains a glycoprotein which reacts with water to form mucous slime. It is likely that strong heavy metal complexing sites in these proteins withdraw lead from seawater and incorporate it into the slime. Our samples of epidermis included the slime coating, and we believe that most of the lead in tuna epidermis is located in epidermal slime. Coombs *et al.*[5] have shown that Ca and Zn are probably concentrated from seawater into epithelial mucus.

Muscle contains quite small concentrations of metabolised lead, probably because of biochemical exclusion mechanisms in the gut membrane. As Table 1 shows, mucus on epithelial tissues in gill filaments do not contain high concentrations of lead, so that it does not seem likely that much lead is adsorbed by gill mucus from seawater and metabolised into fish muscle from gills. As shown in Table 1, for tissues immediately underlying the epidermis, lead concentrations in scales were 1/26th of that in epidermis, while lead in dermis was 1/150th of that in epidermis, but these values may be erroneously high because of cross contamination from mucosal slime during dissection. It seems unlikely that much lead passes through the skin barrier from seawater.

Measured lead concentrations in several sample of commercial canned tuna are listed in Table 1. There is a considerable range in values, one being clearly dangerous to human health. The levels of lead in canned tuna available for human consumption are about 1,000 times above lead levels existing in living tuna. The following evidence shows that this difference in lead levels is common, and is due to contamination by industrial lead.

An interlaboratory reference made of selected high grade fillets of muscle from albacore was prepared by the US National Bureau of Standards (US NBS). The frozen meat fillets were shipped to a commercial food processor, where they were dehydrated, ground, and mixed. Aliquots were placed in polythene bags, and these bags were sealed within lead-soldered vacuum cans for distribution. Lead concentrations determined in this tuna muscle reference are listed in Table 2, together with results obtained for the US NBS bovine liver reference. We consider the bovine liver to be homogeneous in lead on a 0.3 g scale and claim it contains lead within ±2% of the values reported in Table 2 irrespective of the certified value reported by the US NBS (the certified US NBS value is a mean of values determined by different methods, some of which are less reliable than isotope dilution for lead at these concentrations). The considerably wider spread in values obtained for the albacore muscle reference suggests that it is not homogeneous in lead on a 0.3 g scale. The albacore that made up this reference were caught in exactly the same manner, time, and place as the albacore listed in Table 1 (off the southern California coast, July, 1971). The fact that the commercially handled muscle contains 400 times more lead than the same muscle dissected under clean conditions, together with the heterogeneous occurrence of lead in the commercially handled muscle, shows that the tuna muscle reference was contaminated by industrial lead in a commercial food factory by a factor of 400 after it was removed from the sea. This justifies the assertion that canned tuna, representative of that available for human consumption, is contaminated by industrial lead to the extent of about 1,000 times above lead levels in living tuna.

Mucosal slime is not the source of the major part of this lead contamination because it could only raise muscle concentrations by a maximum factor of about 20. Mass balance observations of the distribution of lead in most organs of tuna show that 3% of all the lead in tuna resides at a concentration of about 0.0003 p.p.m. in fresh muscle, while 52% resides in the epidermis at a concentration of about 2 p.p.m. Muscle comprises about 75% of tuna body weight, while epidermis amounts to about one quarter of 1%. The total body burden of lead in albacore tuna is about 0.008 p.p.m.

The following evidence (from ref. 1) suggests that it is difficult, if not impossible, at present for most analysts to determine reliably true values of lead concentrations in marine organisms. The US NBS orchard leaf, bovine liver and tuna references were sent by IDOE for metal analyses to eleven laboratories participating in the IDOE Baseline Studies of Heavy Metals Program. All analysts using atomic absorption and anodic stripping voltammetry techniques were willing to report approximately correct values for the orchard leaf reference, even though it was irrelevant to the problem of analysing lead in marine organisms, because the answer was confidently known and because it contained one hundred and fifty thousand times (44 p.p.m.) more lead than does tuna muscle dissected in a clean laboratory. Not one reported an erroneous value. About half of these analysts refused to report a lead value for the bovine liver reference, while the remainder reported that the lead concentration was less than their ability to measure it (~0.5 p.p.m.). Not one reported an erroneous value. These analysts knew with confidence that lead was about 0.3 p.p.m. in this reference. Most of these analysts refused to report anything regarding lead in the tuna reference. A few reported that lead was less than their measurement limit (~0.5 p.p.m.), while a few reported erroneous lead concentrations that were about an order of magnitude too high. The analysts did not know, before they analysed it, that the lead concentration was 0.4 p.p.m. in the tuna reference.

Since marine organisms can possess soft tissues in close juxtaposition which contain lead concentrations that differ by a factor of 5,000, and since the major mass of live tuna tissue contains such low concentrations of lead that it can be polluted with lead by a factor of 400 before analysis and still contain less lead than most analysts can measure at present, it is clear that a problem exists regarding the evaluation of lead pollution in the marine environment. These difficulties probably extend to the terrestrial environment as well, since it has been recently shown that native field mouse muscle contained only 0.0015 p.p.m. lead in an animal that was known to be 83% polluted with industrial lead, and that mountain freshwater streams contain two orders of magnitude less lead than can be reliably measured by most analysts at present[6].

A fresh outlook regarding lead analyses of biological materials is required. The practical sensitivities and accuracies of atomic absorption and anodic stripping techniques do not appear to be the main sources of difficulty[2]. The real problem appears to be a pervasive lack of understanding of the extent, sources, and ways to eliminate laboratory lead contamination. Substantial improvements could be made if analysts devoted much more care and attention to the evaluation of analytical lead blanks and their reduction and control by the institution of clean-laboratory procedures, together with a realistic scaling up of the quantities of sample lead involved in an analysis so as to substantially exceed laboratory analytical lead blanks.

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Sensitisation of lymphocytes *in vitro* against human melanoma-associated antigens

SENSITISATION of lymphocytes *in vitro* facilitates the study of cellular immune mechanisms under conditions in which the exact input of reactive cells and antigen is controlled. Such systems have been used to generate cytotoxic effector cells against alloantigens[1-3], and more recently against tumour-associated antigens (TAA) in animal models[4,5]. Less work has been done on TAA induction of immune responses using human antigens and effector cells. Golub *et al.*[6] demonstrated *in vitro* sensitisation against antigens present on autochthonous Burkitt lymphoma cells. Since such sensitised cells mediate tumour or allograft rejection reactions *in vivo*[7,8], the *in vitro* 'education' of lymphocytes against TAA is an appealing potential modality for immunotherapy of human cancer. Several preliminary clinical trials have been reported in which 'educated' lymphoid cells were administered to cancer patients[9,10], although the clinical benefits of this procedure remain uncertain. We describe here culture conditions that increase cytotoxic activity of melanoma patients' lymphocytes against melanoma-derived target cell lines.

Dr Hector Sulit of our laboratory established target cell lines from three patients with malignant melanoma and from one patient with osteosarcoma. Control fibroblast lines were established from uninvolved areas of skin or muscle from these patients. The lymphocytes and the cultured cell lines were designated as follows: lymphocytes as 4L, 10L and 14L, cell lines as MLA4, MLA10 and MLA14, fibroblast cultures as MLA4F, MLA10F and MLA14F, and the sarcoma target cell culture as SLA1. The long term breast carcinoma line, BT20, was used in experiments 2 and 4. All other cell lines were used between the fifth and fifteenth passage. All cell lines were free of detectable mycoplasma contamination.

Lymphocytes, usually containing 5–15% monocytes and less than 2% granulocytes, were prepared from peripheral blood samples by Ficoll–Isopaque gradients and were stored in viable condition by slow freezing. For the sensitisation procedure, 2–4 × 10^6 tumour cells were allowed to form a monolayer in a 25 cm^2 Falcon tissue culture flask. Once the cells were firmly attached, the monolayers were treated with 5 $\mu\mathrm{g\,ml}^{-1}$ of mitomycin C and incubated for 18 h. The monolayers were washed and 5 × 10^6 to 9 × 10^6 lymphocytes were added in RPMI 1640 medium supplemented with antibiotics, 15 mM Hepes buffer and 20% pooled normal AB serum. The lymphocytes were sensitised on the monolayers for 5 d, after which they were recovered by washing with warm media. Viable lymphocytes were counted by trypan blue exclusion in a haemocytometer (tumour cells were not viable at this point due to mitomycin). Forty to sixty per cent of the original lymphocytes in culture could usually be recovered. The lymphocytes were washed and resuspended at 1.0 × 10^6 to 1.5 × 10^6 ml^{-1}. Three or more replicate samples of 0.1 ml were tested for ^{3}H–thymidine incorporation and additional aliquots were tested for cytotoxicity. Lymphocyte proliferation was determined by a micro-MLC assay similar to that of Thurman *et al.*[11]. Samples were pulsed with 0.5 μCi of isotope and incubated for 18 h before collection. Cytotoxic activity was assayed by the ^{125}I–iododeoxyuridine (IDU) method of Cohen *et al.*[12]. In this test, lymphocytes are added to ^{125}I–IDU-labelled target cells in Falcon Microtest II plates, usually at an effector: target cell ratio of 100:1 (some experiments involved ratios of 150:1 and 50:1). After 40 h of incubation, the plates were washed to remove lymphocytes and dead target cells. The c.p.m. of isotope remaining in each well indicated the number of target cells surviving the interaction with the lymphocytes.

All experiments had a control culture of lymphocytes cultured for 5 d in the absence of a stimulator cell. Results of the ^{3}H–thymidine incorporation are expressed as a response index (*RI*) calculated by the formula *RI* = Average c.p.m. of sensitised lymphocytes/Average c.p.m. of control lymphocytes.

Results for the cytotoxicity assay are expressed as a cytotoxic index (*CI*) calculated by the formula *CI* = [(average c.p.m. in wells + control lymphocytes) – (Average c.p.m. in wells + sensitised lymphocytes)]/[Average c.p.m. in wells + control lymphocytes].

By calculating the results in this fashion, we could describe only the cytotoxic activity obtained by the sensitisation procedure, rather than the cytotoxicity intrinsic to the melanoma patient's lymphocytes.

In experiment 1, the lymphocytes from melanoma patient 14 were sensitised *in vitro* on autochthonous and allogeneic tumour cells (Table 1). Autochthonous and allogeneic sensitisation produced a small, but significant increase in lymphocyte proliferation. Both sensitised lymphocyte populations displayed increased cytotoxicity against melanoma target cells,

Table 1 Sensitisation of 14L on MLA14 or MLA10

Assay	Effector cells 14L	 14L+MLA14	 14L+MLA10
Average c p m ^{3}H–thymidine incorporated/10^5 lymphocytes ± s e	444±28	864±42	658±25
RI	1 00	1 95	1 48
*P**	—	<0 0005	<0 005
Average c p m retained MLA14 target cells±s e	3,042±149	1,419±101	2,256±97
CI MLA14 target cells	—	0 53	0 26
P	—	<0 0005	<0 005
Average c p m retained MLA14F target cells±s e	845±27	797±42	860±23
CI MLA14F target cells	—	0 06	−0 02
P	—	n s	n s
Average c p m retained MLA10 target cells±s e	1,215±76	770±40	769±80
CI MLA10 target cells	—	0 37	0 37
P	—	<0 0005	<0 005

* *P* values were determined by one-tailed Student's *t* test by comparison of control culture (14L) to sensitised culture, ns, not significant at 0 05 level

but not against fibroblasts Autochthonous sensitisation led to greater proliferative and cytotoxic responses than did allogeneic sensitisation This result was not a consistent finding (Table 2) and may be related to the varying antigenicity in the cell lines, because MLA14 has been our strongest stimulator cell

Four further melanoma sensitisation experiments are summarised in Table 2 Lymphocyte samples from all three melanoma patients were sensitised against the autochthonous tumour line as judged by increased cytotoxic activity In each case, the increased cytotoxic activity was only detectable against melanoma target cells, but not against tumour cells of different histological types or against autochthonous fibroblasts The sole exception was some slight 'non specific' cytotoxic activity of the MLA 4-sensitised 4L against the SLA1 target Thus, the cytotoxic activity generated by sensitisation on autochthonous melanoma cells appeared to be specific for a melanoma-associated antigen Sensitisation on allogeneic melanoma cells resulted in even greater cytotoxic activity against either the allogeneic or autochthonous tumour target cells in two of the three experiments Again, no cytotoxic activity against autochthonous fibroblasts was detected Mitomycin-treated stimulator cells alone were not toxic to target cells, nor did they incorporate ^{3}H–thymidine

Lymphocyte proliferation after sensitisation on autochthonous tumour cells was much more variable than was cytotoxicity Only experiments 1 and 3 showed any significant increased incorporation of ^{3}H–thymidine, while none was evident on 10L sensitised on MLA10 (experiments 4 and 5) Autochthonous sensitisation of 10L was less vigorous in cytotoxic response as well The differences among patients may be related to differences in clinical status or perhaps to the kinetics of lymphocyte proliferation Preliminary evidence indicates that the two lymphocyte functions, proliferation and enhancement of cytotoxic activity, may follow different time courses with the peak of proliferative activity preceding the peak of tumour-specific cytotoxic activity This finding is similar to reports from other investigators[13–15] stating that the proliferative and cytotoxic phases of alloantigen sensitisation can be dissociated Details of the time and dose requirements for sensitisation in our system will re reported elsewhere

The antigens to which the lymphocytes were responding appeared to be melanoma-associated because of the pattern of cytotoxic activity and the requirement for melanoma antigens for sensitisation Sensitisation on autochthonous normal fibroblasts (Table 2, experiments 6 and 7) did not result in significant cytotoxicity against any target cell Lymphocytes from patient 4 were sensitised on autochthonous fibroblasts and yielded no significant cytotoxic activity against any target Similar results have been obtained with 10L on MLA10F and 14L on MLA14F Thus, the sensitisation procedure as used in these studies appeared to require the presence of the tumour-associated antigens, and was not simply the result of the cocultivation of lymphocytes with another cell type We are now looking for culture conditions which may allow autosensitisation against normal 'self' antigens expressed on both the fibroblasts and tumour cells[16] Preliminary experiments indicate that sensitisation on allogeneic sarcoma cells does not result in cytotoxicity against the autochthonous melanoma target, again indicating the requirement for the melanoma antigens

The failure of the lymphocytes sensitised on autochthonous cells to kill autochthonous fibroblasts was an especially important control as both these melanoma cells and fibroblasts expressed HL-A antigens in a quantitatively similar fashion (R Reisefeld, personal communication) Both cell types were equally, or nearly equally, sensitive to effector cells sensitised against the alloantigens by mixed lymphocyte culture (MLC)

Table 2 Sensitisation of lymphocytes *in vitro*

Experiment no	Responder lymphocytes (effector cells)	Sensitising cell	Response index	Cytotoxic index Autochthonous melanoma target	 Autochthonous fibroblast target	 Allogeneic melanoma target ‡	 Allogeneic sarcoma or target ‡
2	14L	MLA14	1 28	0 19*	0 06	0 17*	<0 00
3	4L	MLA4	3 37†	0 43†	—	0 43†	0 22*
	4L	MLA10	7 72†	0 49†	—	0 47†	0 12
4	10L	MLA10	0 57	0 22*	<0 00	0 09	0 00
5	10L	MLA10	0 53	0 39*	0 10	0 28*	
	10L	MLA14	3 68*	0 70*	0 00	0 50*	
6	4L	MLA4F	1 84*	0 16	<0 00	<0 00	
7	4L	MLA4F	0 18	<0 00	0 04	<0 00	
8	D	14L	9 19†	0 54† (MLA14)	0 50† (MLA14F)		
9	G	4L	11 57†				
	(150 1)			0 46† (MLA4)	0 25† (MLA4F)		
	(50 1)			0 21†	0 18†		

* *P*<0 05 by Student's *t* test when compared with control lymphocytes
† *P*<0 005
‡ Allogeneic melanoma target cells were MLA10 in experiments 2 and 3 and 7, MLA4 in experiment 4, and MLA14 in experiment 5 and 6
Allogeneic target cells of different histological types were BT20 breast carcinoma in experiments 2 and 4, and SLA1 sarcoma in experiment 3

(experiments 8 and 9, Table 2) In these experiments, lymphocytes from normal donors D or G were sensitised in MLC with mitomycin-treated lymphocytes from patients 4 or 14 The recovered lymphocytes were then tested for cytotoxic activity against both the melanoma cells and the fibroblasts from the donor of the stimulator lymphocytes It was clear that the fibroblast targets were sensitive to the action of effector cells and were not resistant to lysis

In summary, it is possible to generate a cytotoxic response *in vitro* against antigens that seem to be tumour-associated If such sensitised cells have specific cytotoxic activity *in vivo* as well, they may provide a useful therapeutic agent

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B and T-cell stimulatory activities of multiple mitogens from pokeweed

MITOGENS, which stimulate cell division in immunocompetent cells, are being used in the study of control mechanisms in cellular division and differentiation In general mitogens are specific for one major class of cells To stimulate immunocompetent cells[1 2], most soluble plant mitogens such as concanavalin A (con A) and phytohaemagglutinin require the presence of thymus-dependent lymphocytes (T cells) On the other hand, bacterial lipopolysaccharide (LPS) stimulates the B (thymus independent) class of lymphocytes[3] In contrast, extracts of *Phytolacca americana* (pokeweed) are reported to have mitogenic activities directed toward both T and B lymphoid cells[4-7] A study of the pokeweed system may provide further information on the differences and similarities in the mechanisms by which these two classes of lymphoid cells are stimulated

We have isolated and characterised five mitogenic proteins from *Phytolacca americana*[8], designated Pa-1 to Pa-5 They are all mitogenic for murine spleen cells over an unusually wide range of protein concentrations We now report their ability to stimulate mitosis in different populations of murine lymphoid cells A complete set of data for Pa-3 is not included in this study because of the very low yield of this protein

The mitotic responses of the different lymphoid populations were measured by the incorporation of ^{3}H-thymidine into the newly synthesised DNA The cells (4×10^5 per well) were cultured by the micro method in the presence of the mitogen for 48 h They were then pulsed with 1 μCi of ^{3}H-thymidine and collected 18 h later as previously described[8]

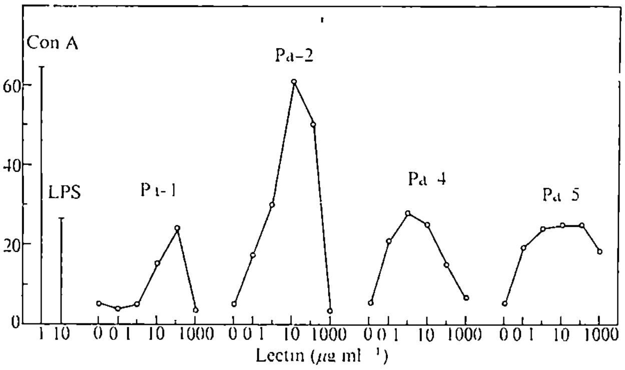

Fig 1 Incorporation of ^{3}H-thymidine by normal spleen cells from BALB/c mice in the presence of the pokeweed mitogens Con A (1 μg ml^{-1}) and LPS (10 μg ml^{-1}) were included as controls for the stimulation of T[1 2] and B[3] cells respectively Results are the geometric mean of triplicate cultures

The dose-response curves of normal spleen cells to these mitogens is presented in Fig 1 Con A, LPS and the four pokeweed mitogens stimulated this mixed population of T and B cells The maximum responses of Pa-1, Pa-4 and Pa-5 were about the same and occurred with concentrations between 1 and 100 μg ml^{-1} Pa-2 induced a considerably higher incorporation, equivalent to that caused by con A, and its maximum occurred at 10 μg ml^{-1} It is unusual for lectins to be mitogenic over such a wide range of concentration For example, con A is mitogenic over a range of a few μg ml^{-1} (refs 4, 9) In contrast, the B cell mitogen, LPS, is active over a wide range of concentration[3]

Figure 2 shows the dose-response curves of thymocytes to these mitogens Incorporation was less than with spleen cells, but the ratios of the maximum stimulated values to the control values were greater The response to LPS was the same as the unstimulated control This is consistent with the absence of B cells and with our observation that less than 1 % of thymocytes stain for immunoglobulin when labelled with fluorescent antibody Con A, the specific T cell mitogen, and all four pokeweed mitogens stimulated these thymocytes Once again, Pa-1, Pa-4 and Pa-5 showed roughly equal activities, about half that of con A, and produced maximal stimulation between about 10 and 100 μg ml^{-1} Pa-2 was the most active mitogen by far, with an activity about three times that of con A These results clearly indicate that the four pokeweed mitogens have stimulatory activities directed toward T cells

To evaluate the mitogenic activities of these pokeweed proteins toward B cells, assays were performed using spleens from TXBM (see legend to Fig 3) mice and spleens from nude mice The dose response curves of TXBM spleen cells to these

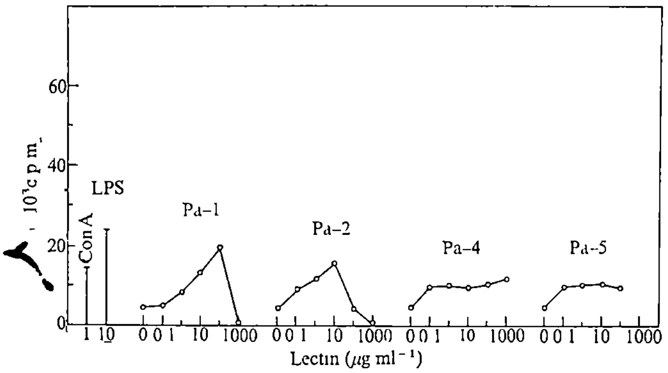

Fig 2 Incorporation of ^{3}H-thymidine by normal thymocytes from BALB/c mice See legend to Fig 1

mitogens are presented in Fig 3 The unstimulated control values were the same as with normal spleen cells (Fig 1), but there was a marked decrease in the response to con A The response of TXBM spleen cells to LPS remained at about the same level as with normal spleen cells Of the four pokeweed mitogens, only the response to Pa-1 remained near the normal spleen cell value The response to Pa-2 decreased to about a quarter, and the responses to Pa-4 and Pa-5 dropped to about one-half of their values with normal spleen cells (Fig 1) The diminished but substantial response to con A may indicate the survival of a population of T cells in these TXBM mice as reported by others The observation that both LPS and Pa-1 were the only mitogens to cause responses similar to those with normal spleen cells suggests that Pa-1 may be mitogenic for B cells as well as T cells The presence of T cells, indicated by the response to con A, makes it impossible to conclude whether or not the response to Pa-1 and the lower responses to Pa-2, Pa-4 and Pa-5 are due to B cell activities on their part or due to residual T cells in the cultures

This ambiguity is resolved by the results with spleen cells from athymic (nude) mice (Fig 4) The unstimulated control values were the same as with normal and TXBM spleen cells (Figs 1 and 3) There was no response, however, to con A, consistent with the absence of T cells, and the B cell response to LPS was increased Of the four pokeweed mitogens, only Pa-1 stimulated these B cells

All these results suggest that in the murine system Pa-1 is mitogenic for both T and B classes of lymphoid cells and that Pa-2, Pa-4 and Pa-5 are mitogenic for only the T class While the trace mitogen, Pa-3, was not included in all these experiments our preliminary results indicate that it has biological properties similar to Pa-2 but is less potent

The differences in the mitogenic specificities of these proteins are directly reflected in their physicochemical properties[8] Pa-1 differs markedly in these properties from the four other pokeweed mitogens Pa-1 has a subunit molecular weight of 22,000, but behaves as a multimer or aggregate during gel filtration in non-dissociating solvents Pa-1 also differs noticeably from the other four proteins in its amino acid composition and has few disulphide bonds In contrast, Pa-2, Pa-3, Pa-4 and Pa-5 are similar to one another in amino acid composition, have many disulphide bonds, and appear to be monomers[8]

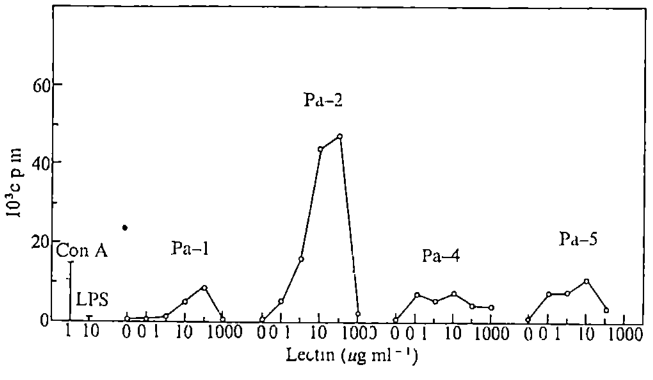

Fig 3 Incorporation of ^{3}H-thymidine by TXBM spleen cells BALB/c mice (4–6 weeks old) were thymectomised, 10 d later they were X irradiated (900 rad) and reconstituted with 7 × 10^6 anti-θ-treated bone marrow cells from littermates The spleens were used 4–6 weeks later See legend to Fig 1

The polymeric state of Pa-1 may be the key to its ability to stimulate B cells It may be that B cell activation requires the interaction of several cell receptors with multimeric stimulants This concept is in accord with the finding that attachment of T cell mitogens such as con A to the bottom of a Petri dish[10] or Agarose beads[6] induces B cell activity Indeed, the attachment of the pokeweed T cell mitogens Pa-2, Pa-4 and Pa-5 to beads induces B cell activity (our unpublished results) The requirement of multiple interactions of the B cell immunoglobulin receptors with antigens[11] and antibodies[12 13] for stimulation has been reported and discussed In a similar way, the multimeric state of Pa-1 and the spatial complex of repeating mitogen molecules attached to beads may react with the B cell to induce mitosis

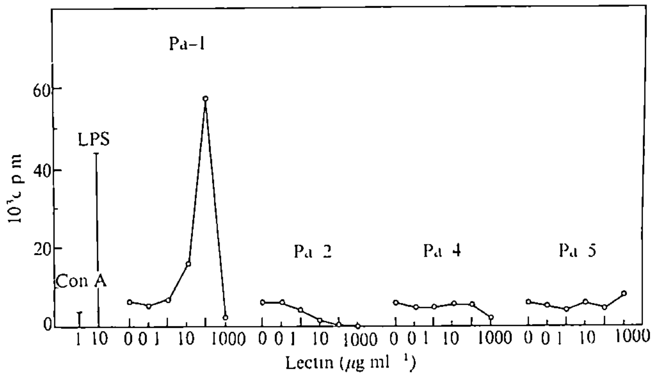

Fig 4 Incorporation of ^{3}H-thymidine by nude spleen cells obtained from athymic mice bearing the *nu/nu* gene on a Swiss genetic background The responses of normal littermates did not differ significantly from the BALB/c spleen cells See legend to Fig 1

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matters arising

Liquid drop model of elastin

WE wish to show how the calorimetric observations of Weis-Fogh and Andersen[1] may be interpreted in terms of the classical theory of rubber elasticity It was observed by Weis-Fogh and Andersen that when, for example, 1 33 mcalorie of work was done on a specimen of elastin immersed in water at room temperature, the heat evolved was 5 53 mcalorie according to the theory of rubber elasticity the heat evolved should be close to 1 33 mcalorie This experiment and others of a similar nature with water–ethanol solutions as solvent lead Weis-Fogh and Andersen to reject the classical theory[2] which they replaced by a two-phase model, the liquid-drop elastomer

In approaching this anomaly we formulated the working hypothesis that the apparent conflict between the theory of rubber elasticity and the calorimetric experiments was due to the erroneous assumption that heat change due to the stress-induced absorption of solvent was negligible If a specimen is immersed in a permeating solvent and elongated then it is to be expected that solvent will diffuse into the specimen so that the heat liberated will depend on the magnitude of the differential heat of dilution, $\overline{\Delta H}$ (ref 3)

The most striking observation of Weis-Fogh and Andersen[1] is the systematic decrease in heat release for the elastin–water/ethanol system as the ethanol content of the solvent increases from 0 to 50% At 20% ethanol the heat release equals the work done, in agreement with the theory of rubber elasticity This can be explained in one way only, if our working hypothesis be correct At 20% ethanol $\overline{\Delta H}$ must be zero below 20% ethanol $\overline{\Delta H}$ must be negative and above 20% ethanol it must be positive

In order to determine the magnitude of $\overline{\Delta H}$ it is necessary to determine three partial derivatives[3],

$$\overline{\Delta H} = -T\frac{(\partial f/\partial N)_{PLT}(\partial N/\partial T)_{PLe}}{(\partial N/\partial L)_{PTe}} \quad (1)$$

in which f is force, P pressure, L length, T temperature and e signifies that the specimen is in equilibrium with a reservoir of diluent from which it has absorbed N mol Of the three partial derivatives only one, $(\partial N/\partial T)_{PLe}$ can be measured easily Both $(\partial f/\partial N)_{PLT}$ and $(\partial N/\partial L)_{PTe}$ can be measured but with greater difficulty Since we required only the sign of $\overline{\Delta H}$ we measured $(\partial N/\partial T)_{PLe}$ and assumed $(\partial f/\partial N)_{PLT}$ to be negative and $(\partial N/\partial L)_{PTe}$ positive This assumption is based on sound *a priori* arguments (the opposite assumption would be extremely rash) and is in accord with observation, for instance Abe and Prins[3] for the system water–polyvinyl alcohol It follows then that $\overline{\Delta H}$ will have the same sign as $(\partial N/\partial T)_{PLe}$

Measurements of $(\partial N/\partial T)_{PLe}$ on purified pig aorta at temperatures between 0° and 50° C, have shown that at 20% ethanol $(\partial N/\partial T)_{PLe} = 0$ Below 20% ethanol $(\partial N/\partial T)_{PLe}$ is negative and above it is positive Thus $\overline{\Delta H} = 0$ at 20% ethanol and at this composition the system behaves as a classical elastomer in the calorimetric experiments Full details of this work will be published in due course

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The Gibbs Fracture Zone

I WISH to comment on the idea that the Gibbs Fracture Zone may be a westward projection of the Hercynian front into North America Cherkis *et al*[1] have argued in favour of a correlation between the Gibbs Fracture Zone and the late Palaeozoic Hercynian orogenic front

Geophysical evidence[2] indicates that the Gibbs Fracture Zone extends from the Newfoundland continental margin near 52°N 47 5°W to a point west of Ireland, near 52 25°N 16°W Off Newfoundland the fracture zone seems to be associated with a 200 km sinistral offset of the continental edge[3]

If Wilson's[4] theory for the propagation of oceanic fracture zones from old lines of weakness in a formerly contiguous continental crust is applicable here, then an equivalent offset should be present in the European margin Such an offset has not, as yet, been found It is clear that one does not exist in the western margin of Porcupine Bank as implied by the argument of Cherkis *et al*[1] A possible site is near the mouth of Rockall Trough, but the shape of the continent–ocean boundary in this region is not well known

Despite this lack of knowledge about the eastern end of the Gibbs Fracture Zone, it is instructive to follow Wilson's theory and see which predrift continental lineament aligns best with it To do this, I have drawn Palaeozoic structural features of Newfoundland and the British Isles on a new reconstruction of the North Atlantic continents (Fig 1) This reconstruction is more consistent with current knowlege of the structure of the Atlantic Ocean basin than is the reconstruction of Bullard *et al*[5] which was apparently used by Cherkis *et al*, after Hurley The trends of the structures in Ireland are taken from Kennedy *et al*[6] The westward projection of the Hercynian front has not, to my knowledge, been defined by geophysical work, but in the Celtic Sea to the south, a west-south-west Hercynian trend may exist[7] It is therefore possible that the front trends west-south-west In the case of the Caledonoid lineaments there is some

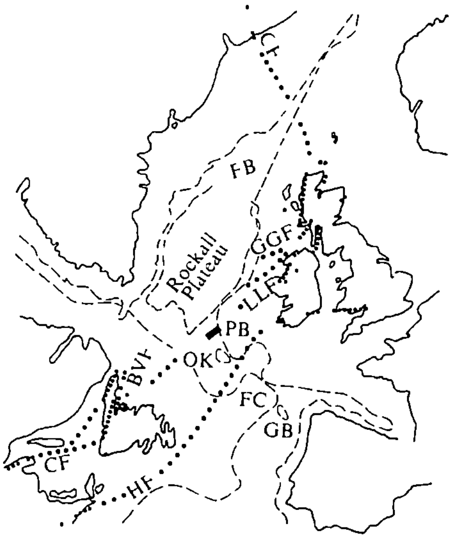

Fig. 1 A reconstruction of the North Atlantic continents (from Scrutton, R A, unpublished) showing Palaeozoic structural lineaments Closely dotted lines, well known trends, widely dotted lines, less well known or conjectural, solid rectangle, the position in which the Gibbs Fracture Zone developed during North Atlantic opening, FB, Faeroes Bank, PB, Porcupine Bank, OK, Orphan Knoll, FC, Flemish Cap, GB, Galicia Bank, CF, Caledonian Front, HF, Hercynian Front, GGF, Great Glen Fault system, LLF, Leck-Leannan Fault, BVF, Baie Verte Fold

magnetic evidence for a general westerly projection towards the outer edge of Porcupine Bank[8] Clearly, correlation of the Gibbs Fracture Zone with any of these structural trends, in particular the Hercynian front, is tenuous

Trends in and around Newfoundland are taken from refs 3 and 6 At present, there is no evidence from Newfoundland of any thrust that can be considered as the equivalent of the Hercynian thrust front of New Brunswick and the British Isles The magnetic anomaly lineations, which Cherkis *et al* suggest indicate the Hercynian Front, in fact overlie a zone of mafic and ultramafic intrusions of Caledonian age[9] and parallel the Caledonoid Hermitage Flexure The Hercynian Front is therefore taken south of Newfoundland to meet the continental edge between Orphan Knoll and Flemish Cap The Caledonian structural trends apparently continue north-eastwards from Newfoundland to the continental edge near the Gibbs Fracture Zone[3], it could be one of these rather than the Hercynian Front that best correlates with the fracture zone

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DRS CHERKIS, FLEMING, and MASSINGILL REPLY Scrutton's argument that no offset has been found to link the Gibbs Fracture Zone to the European continent is certainly valid Likewise, his proposed site for the existence of the offset "near the mouth of Rockall Trough" deserves attention We did not, however, suggest[1] that the Gibbs Fracture Zone is represented by the Hercynian Front, but rather that the Hercynian Front provided the zone of weakness in the Proto-Atlantic Ocean, along which a transform fault evolved on the Mid-Atlantic Ridge after the Atlantic [re]opened

Using seismic profiling we have determined that the Gibbs Fracture Zone can be traced to 16°W At that point, the relative proximity of the Porcupine Bank means that the fracture zone cannot turn northward into Rockall Trough The lineation trend of the Hercynian Front in southern Ireland cannot be ignored Projecting this trend across the Porcupine Bank from the Irish coast to the Gibbs Fracture Zone (admittedly, a 180-mile gap) the match is too perfect to be mere coincidence Gray and Stacey[2] found gravity complexities over the Porcupine Bank and Bight that show that positive free-air anomalies terminate abruptly along the projected frontal line Vogt and Avery (Fig 16 of ref 3) showed that negative magnetic anomalies occur along the projected frontal line, interrupting the north–south lineations

Regarding the North American link, Grant[4] showed the Gibbs Fracture Zone coming into the continental margin, and crossing a fault plane We projected the fracture zone through the fault plane and through central Newfoundland, *en echelon* with, or coinciding with, the Caledonian Front An alternative suggestion is that the fault plane may include a strike-slip line, offsetting the Gibbs Fracture Zone to the south through Flemish Pass, and then continuing its easterly course through Nova Scotia and New Brunswick Unfortunately, magnetic measurements do not confirm this, they indicate a more northerly pattern than Scrutton implies from his Fig 1, that is, across Cabot Strait, rather than toward Chedabucto Bay and Nova Scotia Furthermore, Rast and Grant's[5] observations of Hercynian structures in New Brunswick seem to confirm the magnetic trend

The answers to these questions can be solved only with more detailed geophysical studies of the areas

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External origin for lunar rilled craters and the mare basins

FLAT-FLOORED craters on the periphery of the lunar maria often contain rilles Because of their location, Whitford-Stark[1] has proposed that these craters are of internal origin and that the mare basins also must be so The assumption that "rilles in all internally rilled craters formed at about the same time as the crater floor" is central to Whitford-Stark's theory Yet, Repsold, Vasco da Gama R, and other ancient, broken craters contain sharp rilles, which often cut mare albedo floors The presence of mare material (lava) on the floors of ancient pre-Imbrian craters shows that the floors, and therefore the rilles, formed after the craters

Quantative support for partial flooding of rilled craters by lava comes from Whitford-Stark's depth–diameter diagram which illustrates that the majority of rilled craters are shallower than normal 'eumorphic' craters Additional evidence of lava fill is provided by his observation that the floors of rilled craters generally have a lower crater density than the floors of non-rilled craters of the same stratigraphical age Thus, the difference between rilled and non-rilled craters need not result from different origins, but rather, may depend upon whether they have been flooded by lava Similarly, the wide scatter of depths of craters of Imbrian age may have resulted from variable depths of lava fill In this context it may be noteworthy that nearly 50% of the craters plotted on Whitford-Stark's depth–diameter diagram are of Imbrian age, that is to say that the craters were formed when magma was abundantly available

There is an alternative to the hypothesis of internal origin for rilled craters Mare basins resulting from gigantic impacts[2] profoundly fractured the lunar crust, creating conduits to the surface for magmas which were extruded approximately 200 to 800 Myr later[3] Craters, predominently of impact origin (ref 4, and Wood, C A, unpublished), that formed near the basins were often flooded by magma rising up basin fractures Rilles could then form, as Whitford-Stark suggested, by contraction of cooling lava ponded within the craters, or by the fracturing of tholoid domes Rilled craters could also form along other zones of structural weakness—such as the Rheita valley[1]—which would provide fractures for magma ascent Thus, although the formation of rilles within craters is a volcanic process, the craters themselves, and the basin fractures, most likely result from impact

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reviews

Neurones or semiconductors?

On Machine Intelligence By Donald Michie Pp xi+199 (Edinburgh University Edinburgh, April 1974) £2 50

THIS book is a collection of "semi-popular essays written over the past ten years or so" The author's criterion for inclusion is stated with candour "If I enjoyed writing the essay in the first place, and if now I enjoy re-reading it, then I have put it in, otherwise not" The book contains a great deal of repetition—the same tables appear in several different articles, and the same themes occur over and over again with little variation in their treatment Some articles even contain quotations from others

Since the essays were originally intended to be popular, the only work that is described in detail is of a very simple nature such as the author's device for learning to play a good game of noughts and crosses by trial and error Games playing programs receive adequate coverage but there is little attempt to describe the principles behind more recent programs designed to analyse scenes or to understand natural language Readers are told that "powerful new programming aids, such as the PLANNER, QA 4, and CONNIVER languages have come into play", but they are not told what it is about these languages that makes them an advance on previous information processing languages The author has considerable expository skills, and within the limits he sets himself the writing is lively and clear It is a great pity that he has not sat down to write from scratch a systematic introduction to artificial intelligence for the general scientific reader such a book is badly needed and there are few people as well qualified to write it as Donald Michie

The book makes two major claims Michie asserts that developments in artificial intelligence, or as he prefers to call it machine intelligence, will bring about a technological revolution According to "experts", robot chaffeurs and computer psychiatrists will be commercially available by the year 2000 though it will take a further 10 years before automated "general factotums" come off the automated assembly line It may or may not be technically feasible to produce machines that can make as intelligent a use of a wide range of knowledge as do chauffeurs and psychiatrists Leaving this problem aside, there are two arguments, not considered by Michie, which suggest that machine intelligence may not have such a wide role in human affairs by the end of the century

First, in automating a given system, it is often best to redesign it so as to obviate the need for any application of intelligence Automatic pilots do not function in an intelligent way they land planes by flying down a radar beam, and this slavish procedure circumvents the complex perceptual processes used by human pilots It will probably be cheaper and safer to redesign road transport systems in this sort of way, rather than to provide robot chauffeurs that will have to make the same kind of intelligent decisions as a human driver

Second, where automation by the use of unintelligent machines cannot be achieved, as presumably in the case of the psychiatrist, human intelligence is likely to remain for a long time to come a cheaper method of doing the job than the use of large computing systems I once estimated that the cost of a neurone in the brain of a young professionally qualified adult was about 0 00004 new pence This estimate includes the programming costs for the whole system and it is orders of magnitude cheaper than the cost of a Japanese semiconductor The extent to which artificial intelligence makes a contribution to technology depends on cost-effectiveness, and even if the problems of making intelligent systems were solved, it is by no means obvious that they would be the cheapest means of undertaking tasks requiring intelligence

Michie's second claim is that machine intelligence is likely to provide new insights into the working of the human mind, and this assertion is perhaps less disputable Until recently, psychologists did not have a language in which they could couch well formulated theories of how complex information processing tasks are carried out Computer languages provide the appropriate symbolism and the digital computer itself provides a method of rigorously exploring the consequences of a given theory It is perhaps unlikely, however, that programs that simulate the details of the way the brain operates will be written merely by reflecting on the best way in which to program a computer to undertake a particular task It will be necessary also to take into account the experimental evidence on how the brain actually does process information Michie (page 123) asks his readers whether, when parsing a grammatically ambiguous sentence, "you generate the alternative parsings, subsequently to be eliminated by semantic hindsight Of course you don't We can be sure that we do not do this consciously" But recent psychological evidence indicates that at least in some cases we do generate different interpretations at an unconscious level Only one of these may be selected as a result of semantic disambiguation, and it is only the results of such selection that appear in consciousness The study of the mind from an armchair or a programmer's stool contains dangers of this sort, and it is likely that our understanding will be best forwarded by using the computer as a tool with which to construct and to explore theories, at the same time being careful to base the theories on the best experimental evidence available

N S SUTHERLAND

Teaching the heart

Universities Between Two Worlds By Roy W Niblett Pp viii+179 (University of London London, June 1974) £2 85

ARE we, in the Western world, at a watershed of civilisation, "between two worlds"? Professor Niblett believes that we are, and in this book he spells out what he regards as some of the consequences for universities His theme is familiar the growing disenchantment with the emphasis which universities put upon the cognitive, the rational, the analytical approach to phenomena, the insufficiency of this approach to solve the problems of contemporary society, the need, therefore, for universities to provide experience in "sensate culture", to ask questions about ends as well as means, about values as well as facts, to engage (as Niblett puts it) the "individual mind and heart" in the process of higher education It is a plea to university teachers to impart not just knowledge, but wisdom, to their pupils

That Western man is deficient in wisdom and has, if anything, a surfeit of knowledge (much of it useless) is a proposition few would challenge But that universities are the institutions to remedy this deficiency is another matter Niblett believes that they are, and the value of this thoughtful essay is that the reader must decide whether he accepts Niblett's belief or not

His diagnosis of higher education (he has in mind particularly Britain and America) is that it has tried to break away from the pursuit of narrow specialisms, and has failed Liberal studies, joint honours schools, the redrawn 'map of learning' in the new universities, the Harvard red book on general education all have led to disappointment The causes of disappointment include the inflexibility of discipline-oriented teachers, the persistence of competition in examinations, the lure of professionalism, and pedantry among those who teach the humanities "The choice before the humanities is clear either to pretend to be sciences or to acknowledge without shame the experiencing and imaginative elements within any essential study of them " The word 'feelings' occurs often throughout the book, feelings must be recognised, identified, and incorporated into the academic experience

In his courteous and gentle way, Niblett makes some highly controversial assumptions which some of his readers will challenge First, are we really between two worlds? Is this a watershed similar to the great divide between Galileo and the Schoolmen? Many will question this assumption The scientific approach to nature and to man still succeeds superbly in answering scientific questions, it was never designed to answer other sorts of questions Nor are the other sorts of questions novel, needing new techniques As Niblett himself suggests, you do not "decide to love or trust another human being", so case studies in decision making (a popular form of broader education) do not embrace some of the essential human activities But societies have been concerned for millenia with love, trust, and the whole spectrum of moral values

The burden of Niblett's message is precisely the same as Walter Moberly's, written 25 years ago, and it is a response to the same dilemma The only difference is that in 1949 only dons appreciated the dilemma, today students appreciate it too The dilemma is that universities, in common with madrasas in the muslim world and the ancient Buddhist seminaries of India, have for six sevenths of their history been religious institutions, resting on a foundation of faith, commanding a consensus of values It is only since University College, London, was founded that the formal link between faith, morals, and learning was broken Both Niblett and Moberly believe (though they approach the issue from different directions) that universities should adapt themselves to the consequences of this severance But some readers will ask should they? There surely is a case for differentiation of functions among institutions When the church tried to meddle with science in the 19th century, it was religion, and the sensate culture associated with it which suffered If the universities now try overtly to meddle in the non-cognitive sphere, important as it undoubtedly is, is there not a risk that they will prove incompetent (professors are no longer elected on grounds of virtue or wisdom) and that the university's unique function, to teach the art of rational analysis, will be eroded? If the purpose of the book is to stimulate reflections of this kind, it deserves to be widely read

Niblett himself admits that there is no clear way to teach the sensate culture And indeed some of us who are close to students doubt whether they are as impoverished in non-cognitive experience as he supposes Not enough of them find, as he puts it, "a sense of values in an age of facts" This has not been my experience I find more concern with moral issues, more compassion, and a livelier (if puzzling) commitment to the sensate culture among today's students than among those who were students when Niblett and I were undergraduates I wonder whether (perhaps) they are getting on quite well, thank you, without our help It is a humiliating admission for an academic to make after a lifetime in universities, but I, for one, would admit that despite all our anxious design of curricula and the student environment, most students get from universities not what we plan to give them but what they come to find Over their morals and personal values we cannot do in three years what home, school, and church may have left undone in the previous 18 years Over their adolescent intellects we can do a lot we can open the doors of the mind

Eric Ashby

Extended scope of NMR

NMR of Paramagnetic Molecules Principles and Applications Edited by G N La Mar, W DeW Horrocks, jun , and R H Holm Pp xv+678 (Academic New York and London, December 1973) \$42, £19 25

Although the effects of small amounts of paramagnetic impurities on nuclear resonances of diamagnetic solid or liquid samples were discussed in some of the earliest work on NMR in bulk matter, it was not until 1956 that direct observations on the paramagnetic crystal, MnF_2, by Shulman and Jaccarino were described and interpreted in terms of chemical bonding Since then it has been realised that although the powerful electronic magnetic moments in paramagnetic substances induce strong nuclear relaxation in neighbouring atoms in the same compound, the effect is often none the less weak enough to allow a great deal of the information in the nuclear resonance spectrum to be obtained The modulation of the nuclear resonance by the paramagnetic moment turns out to be of the greatest importance, and measurements of NMR spectra of paramagnetic substances have extended in a most dramatic way the application of NMR to such diverse fields on the physical chemistry of transition metal complexes and the structures of enzymes

Professors La Mar, Horrocks and Holm have succeeded in persuading an authoritative group of authors to set out the theoretical bases of this important area of research and to review the range of applications which have been explored up to 1973

The first three chapters by Jesson, Swift and by La Mar, comprising about one fifth of the book, give an excellent account of the basic theory and are so clear and well set out that they will be of great value to the beginner who already has an understanding of the principles of magnetic resonance, as well as to the researcher needing a convenient summary The chapter by Jesson sets out the theory of the paramagnetic shift, including a very clear account of the Kurland and McGarvey general treatment This is illustrated by some examples and at the end of the chapter there is an appendix on density matrix theory The second chapter by Swift deals with the effect of the paramagnetic moment on relaxation times This is a very good and concise exposition of the various effects needed to interpret the spectra In the third chapter the theory of the delocalisation of spin density is set out in detail

The remaining thirteen chapters review the applications of these principles to an extraordinary range of chemical studies including spin distribution over organic and organometallic compounds, stereochemistry, kinetics of intramolecular rearrangements, equilibria in solution, and applications to biochemical studies These review chapters present illustrative examples which are worked out in some detail, for an understanding of which a careful study of the first three chapters of the book is a good preparation

This is a most valuable book for the many research workers who could make good use of measurements of this kind, though the sheer range and versatility of the problems described will be daunting to a beginner

R E Richards

Peripatetic naturalist

Linnaeus's Öland and Gotland Journey 1741 Translated from the Swedish edition of 1745 by Marie Åsberg and William T Stearn. Pp viii+220 (Academic: London, Linnean Society of London, February 1974.) £6, $17.50.

In 1741, at the age of thirty-four, Carl Linnaeus was already an eminent naturalist and professor of medicine when he set out to survey the natural resources of the Baltic islands of Oland and Gotland. Taking with him six male companions, he travelled at the behest of the Swedish Parliament, which was intent on economic recovery through the exploitation of domestic raw materials. While familiarising himself with the natural history of the islands, Linnaeus was instructed particularly to look for plants suitable for making dyes, or with pharmacognostic value, and for clay or earth suitable for the production of such things as pottery and pipes. He was also required to keep an accurate diary, which was pubished in 1745 and is now made accessible to English speaking readers by Drs Åsberg and Stearn in their enjoyable translation. The style is that of daily field notes that have been tidied up grammatically, but not turned into a continuous narrative. Although not competing as bedtime reading with the picaresque novels of the period, the diary provides some interesting insights into the observational methods and common sense attitudes of the man who is remembered chiefly for giving the world the binomial system of nomenclature for plants and animals.

The members of the expedition left Stockholm on May 15, and, travelling on horseback, reached the coastal town of Kalmar on May 27. After waiting in vain for the weather to improve, they embarked on the ferry for the three-mile passage to Oland, hastened by two cases of spotted fever in the house where they had stayed. By July 27, when they returned to the mainland, they had traversed much of this narrow island, as well as Gotland, on which they spent thirty-four days. On the latter, the diary disdainfully records, there were no persons of standing except for two judges and a captain.

Linnaeus was well pleased with the journey. He recorded a hundred plants then unknown in Sweden, including several with pharmaceutical uses, such as *Ruta muraria* (now known as *Asplenium ruta-muraria*) which previously had been imported.

Finding woad (*Isatis*) growing in abundance on Klasen, a small island near Gotland, he recommended that it would be worthwhile to sow the species more extensively. In other places the extraction of dye from plants was recorded assiduously for the parliament. Usually leaves or roots were boiled together with yarn, which was subsequently treated with an alkaline concoction to fasten the colour.

By such a procedure, yellow dye was obtained from burmarigold (*Bidens tripartita*) and juniper moss (*Cetraria juniperina*), brownish red from *Origanum vulgare*, a lemon colour from apple bark and dark brown from soot. But no use had been found for the red colour of the rotting seaweed that covered some of the shores of Oland, and "gave off such a stench, as if ten horses had been roasted in the sun". Seaweed, however, was put to good use in other places, as manure and for thatching roofs.

Local remedies were found for all manner of ills. These included the moss *Sphagnum palustre* which was cooked in beer and laid on swollen legs, grasshoppers that spat out a black liquid, causing warts to disappear, the root of *Valeriana officinalis* which was used for hysteria (Linnaeus failed to record how), and *Solanum officinarum*, ground up with spiders web and rancid pork to treat whitlows.

While the expedition was at Forosund on Gotland the farmers left their fields and fences to indulge in feasting and dancing. Here Linnaeus drew a picture of a seamless bagpipe made from a seal's stomach, with the modulator placed in the pylorus and the base inserted into the oesophagus. Seals (*Phoca vitulina*) served other purposes in the economy of Gotland and there are several accounts of hunting methods. The beasts were caught in nets and either harpooned or bludgeoned on their very sensitive noses. Seal lard was sold, the meat was eaten, and the fresh fat could be used instead of butter for frying pancakes. Porpoises were sometimes caught in the nets accidentally, and Linnaeus thought it quite remarkable that nobody had developed the art of trapping them, for their lard was as useful as that from seals.

Interspersed among meticulous accounts of this sort, and descriptions of fences, corn fields, runic stones, flora and fauna, Linnaeus provided notes on local superstitions. This he did "more to entertain my reader than for any other reason". On mainland Sweden the rational professor was not impressed by the belief that when a farmer's cow died he could transfer the bad luck to his neighbour's cattle by burying the carcase in the neighbour's field or dung hill. Such beliefs "and infinitely much more of similar rubbish" were dismissed as relics of heathen and popish times. Linnaeus' prescription for the eradication of such things was to instruct future theologians well in natural history and natural philosophy, since nothing served so well to
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MARY D

Scientists in the world

Towards a Political Sociology *Science*. By Stuart S Blume. Pp xii+288 (Free Press: New York; Collier-Macmillan: London, April 1974.) £4.95.

Like all really good empirical studies in the human sciences, this one is addressed to a theoretical problem. That is, the nature of the scientific community. The author argues that, for sociological analysis, it must be considered as both involved in national political life and also internally differentiated by power relations between elites and others.

In the post-Rothschild epoch of fully industrialised science this thesis is hardly likely to cause outrage among working scientists. But it does represent an important development in the sociology of science. Lest natural scientists dismiss it on that ground they should recall how naive and short-sighted were the dominant assumptions of scientists' own common sense about the social activity of science all through the postwar period.

In the sociology of science, debate is still organised around the ideas of Robert K Merton. We can distinguish three phases of 'Mertonism', a set of received research paradigms much simpler than the contemporary enquiries by Merton himself. At the beginning was the attempt, through the 'four norms' and the 'ethos' of science, to identify the implicit value system of scholarship with that of the most enlightened democracy, political and economic. In the post-war period Mertonism shifted to the motivational machinery by which men in this idealised scientific community are induced to merge self interest with the common good. Recently, more realistic details have been introduced, exhibiting the community as a geronto-aristocracy, and seeing how well this social structure works.

Even this 'late Mertonism' imagines a community of 'pure' science, doing its own praiseworthy thing in a social vacuum. Blume contends that such a model is drastically over-simple. His main thesis is that the élites in science are maintained in power partly by their external connections and that they e their recruits partly by criteria than scientific merit It is then an ely open question, left as such by ne, whether this arrangement is the t possible The reader may, if he shes, draw the further conclusion at the government of science is ripe or political reform

It would be misleading to represent the book as structured tightly around this particular polemical thesis Rather, there is a broad survey, extending into such unaccustomed fields as unionisation and 'politicisation', but everywhere considering science as one institution among many, whose functioning involves 'politics' in every sense of the term Even where Blume deals with topics traditionally considered as 'internal' to the scientific community, as organisations and 'social control', he unromantically invokes relations of status, authority and power as the basic explanatory principles The material on "scientists and government" presents few methodological problems

Oddly enough the concluding chapter on "innovation and society" rings slightly off-true The case study here, fluoridisation, is of great systematic interest for the sociology of knowledge and control, but it is somewhat outside the main trend of the book, if nothing else than because the 'science' involved is medical (which remains peculiarly remote, intellectually and socially, from the central physical sciences) It may be that in spite of his sympathetic treatment of radical science groups, Blume has mised one crucial feature of the present connection between science and the lay public This is the major part played by non-scientists, notably critical journalists, who muckrake establishment technology from the social and environmental points of view Even if their excesses of zeal and haste do occasionally produce errors of fact and method even greater than those of their technically qualified opponents, they are now a permanent part of the scientific scene Their influence on present recruits to science is not to be overlooked

Considering this book as a contribution within the sociology of science, we may see it as opening a new phase, a final liberation from the Mertonian paradigm Being empirical political sociology, it inevitably changes the terms of the debate away from models of a 'scientific community' towards an examination of a quasi-profession that depends on the state and industry for its existence Sociologists of science no longer need to be tough-minded in demythologising 'science', the simple facts of life about this sector of production render myths, models and ethos equally irrelevant

It will be very interesting to see whether there is an effective reaction against this tendency If not, we can mark one of the most important changes in the political sociology of science viewed historically For the ideology of science which (*pace* Blume) has a significant history extending back a couple of centuries, is built into beliefs and practices of many other spheres of activity, the term 'social science' can indicate this An impairment of the functions performed by that ideology, within science as well as externally, will produce new phenomena quite as interesting as any studied in this present excellent survey

J R RAVETZ

First map of the Moon

Thomas Harriot Renaissance Scientist Edited by John W Shirley Pp x+181+4 plates (Clarendon Oxford, Oxford University London, June 1974) £6 50

THIS book consists of seven varied essays, which are based on papers read at a symposium held at the University of Delaware on 5-7 April 1971 The editor explains in his preface why we should take an interest in Thomas Harriot (1560–1621) "Though recognised by his contemporaries as England's most profound mathematician, most imaginative and methodical experimental scientist, and first of all Englishmen to make a telescope and turn it on the heavens, Harriot had not prepared his work for future generations" This neglect of self-advertisement was doubtless produced partly by the superstitions of the age that could pillory an astronomer, and partly by the need to keep as state secrets the new techniques in instrumental navigation, the means by which England might survive economically He did publish, in 1588, his account of a trip with Ralegh to Virginia, and is consequently now known among historians on both sides of the Atlantic as a pioneer, natural historian, ethnographer, and as one who made a systematic study of the Algonquian language But, as a scientist, he is still the Cheshire Cat's grin

It becomes apparent that Harriot knew the sine law of refraction before Snel and Descartes, and was the first man to draw a Moon map with the aid of a telescope His earliest known telescopic observation of the Moon, on July 26, 1609 (OS) at 9 p m , was made nearly three weeks before Galileo presented his telescope to the Venetian Senate, of which instrument Harriot could have had no knowledge whatever Again, he may be given priority in the telescopic observation of sunspots, first recorded on December 8, 1610

The trouble comes when one assesses the significance of Harriot in the history of science As his pupil and friend Sir William Lower wrote on February 6 1610 "yet too great reservedness hath robbed you of those glories it is possible by too much procrastination to be prevented in the honour of some of your rarest inventions and speculations Let your country and friends enjoy the comforts they would have in the true and great honour you would purchase yourself by publishing some of your choice works" In this connection, Dr North, in chapter 7, reminds us of Arago's "règle aux historiens des sciences", which was public utility, implying prompt publication There is no doubt, however, that Harriot contributed to new techniques in navigation and in the training of navigators

The present volume contains Professor Rosen on the intellectual background, Professor Shirley on Harriot's service with Ralegh, Professor Quinn on the Virginian expedition, Dr Pepper's highly technical paper on mathematical navigation, Dr Tanner on associates of Harriot, with a gratuitous attack on Henry Stevens (1819–86) who found Harriot's will, Professor Jacquot on the new natural philosophy, Dr North on Harriot's ten telescopes and the earliest observations made with them These papers show the signs of their symposium origin The same quotation may appear in different essays, and the references at the ends of the chapters (difficult of access here, why not have them in the proper place at the foot of the page?) and modes of citation are not made completely uniform The nine-page bibliography lists the primary and secondary manuscripts, books published around Harriot's time, and modern studies relating directly or tenuously to Harriot The Clarendon Press should have been more careful over consistency in the references (ibid is in italic or roman, journal volume numbers are in arabic or roman), in ensuring that the "selective" index always led to the correct page, that the plate captions were properly positioned, and that type had not slipped

As Professor Shirley explains, the study of the contemporary evidence, household accounts of the Northumberlands, the public records, the Harriot manuscripts, has not been completed This book of essays is, then, an expensive trailer for the main feature still to be shot One can query the value of publishing this collection in book form, thus giving it permanence What is needed first is a detailed description and assessment of the Harriot manuscript material Only when this was been done can his significance be evaluated

G L'E TURNER

Detecting nuclear particles

Nuclear Electronics By P W Nicholson Pp xiv+388 (Wiley London and New York, April 1974) £8 75

THIS book is aimed specifically at physics graduates needing to use nuclear detectors The first two chapters are introductory accounts of the principles involved in detecting nuclear particles and of the basic ideas of pulse circuits, these chapters are provided on the assumption, which unfortunately is probably correct, that most undergraduate courses contain very little on these topics The next three chapters give an account of the general problem of the amplification of nuclear pulses and the methods of minimising the effect of noise This is the most attractive part of the book as the author gives particularly clear explanations of the problems which arise and the methods used to solve them The remaining chapters deal with discrminators, digitising techniques, for both amplitudes and time differences, and various more specialised circuits In these sections it is inevitably more difficult to provide any connected chain of argument but the treatment is still reasonably uniform any theory is given first, the various difficulties and their solution are clearly explained and a well chosen selection of recently published circuits is given as illustration

This is essentially a text book rather than a practical guide Brief accounts are included of the operation of some semiconductor devices, but the whole subject of component characteristics and limitations is omitted and there is hardly any mention of practical 'engineering' problems such as interference, effects of stray capacitances or long-term stability

I think the author has been very successful in carrying out his purpose but unfortunately a detailed theoretical understanding is not sufficient for the experimental physicist, whose problems in practice are of two sorts First, given a large budget, he has to ask whether commercially available equipment will have the necessary performance, this book will certainly help him to understand manufacturers' specifications but considerable skill and experience in electronics is necessary to solve those problems for which equipment is not available Second, and especially for those with inadequate budgets, it is necessary to know how to build circuits with a performance which, though less than 'state of the art', may still be adequate for a particular purpose So it seems a pity that Dr Nicholson, by concentrating on the best published circuits, gives little indication of what may be achieved more simply For example, it would have been worth stressing how easy it is, even for the non-specialist, to use digital integrated circuits in constructing counting equipment

Despite such omissions, this book will be very useful to anybody working with nuclear counting systems I noted only a few errors or misprints and the author is to be particularly commended on the clarity of his explanations and of the diagrams J C BARTON

Counting heads

A Workbook in Demography By Roland Pressat Translated by E Grebenik with C A M Sym Pp 292 (Methuen London, May 1974) £7 90

THE age of the population explosion and its implications for society and for the world's resource base has attracted students in several disciplines to examine demographic problems This interest is catered for by a growing general literature and by regional and case studies, but the techniques and methodology of demography have been generally somewhat neglected Many postgraduate students find a need to provide their own basic training and regret the absence of straightforward, general textbooks that explain not only simply concepts but also the practical mechanism of calculating data from raw statistics, illustrating where relevant the major pitfalls for the unwary Few are as well qualified to undertake this task as Professor Pressat, a mathematician by training, who has done much to define the aims and methods of the quantitative approach to population study He has provided us with just such a textbook as described above, setting up problems, supplying suitable data and offering means of solution and explanation of significance

The "exercises"—for this is truly a workbook—have been selected to present real life situations The examples are spread widely over the confusing variety of situations that confront the beginner and the author brings to them an understandable order and relevance For the more advanced student, he has sought to provide examples close to the situations faced by research workers Not all the exercises are mathematical several depend on descriptive interpretation Nevertheless, using his early training, he makes the mathematics intelligible to those with only modest numeracy Although designed for students in the University of Paris and consequently using substantial amounts of French data, the author has nevertheless selected from a wide range of countries, both "advanced" and "developing", and he has included some most interesting Soviet material

The topics are grouped conceptually, starting with the concept of a population The reader is then taken through the measurement and comparison of mortality and some of the main problems of such difficulties as the misstatement of age The exercises include not only contemporary situations but also the techniques of calculating data for past conditions Anomalies, wherever they occur, are explained at length and in his answers the author makes some interesting comments on contemporary problems For example, the stress of modern urban living may kill the weaker older people who survived infancy through the advances of modern medicine, whereas in earlier times they would have been winnowed by natural selection in childhood Nuptiality, disunion (separation?) and re-marriage are dealt with in a similar manner and are followed by examination of fertility, where material is drawn from North American and African experience and used in comparative studies The final section covers ageing population, infant mortality, birth order and replacement It is a pity there is nothing on migration

Treatment is thorough and wherever necessary basic techniques are explained The student will find the many valuable formulae most useful and the author explains how to use fragmentary and unsatisfactory data by skilful manipulation This is indispensible as a reference book for anybody interested in population

R E H MELLOR

Not so fast

Chemical Kinetics Homogeneous Reactions By N M Emanuel' and D G Knorre Translated from Russian by R Kondor Translation edited by D Slutzkin Pp xii+447 (Wiley New York and Toronto, Israel Program for Scientific Translations Jerusalem, February 1974) £8

THIS is a soundly written book with a broad coverage of topics It is, however, mainly concerned with work up to and including the early transition state era As a succinct example, the work of Michael Polanyi on energy surfaces is described, but not that of his son John Polanyi All this gives the book an oddly out-of-date flavour Short references to flash photolysis, electron paramagnetic resonance and so on indicate the updating efforts of the second Russian edition (Moscow, 1969) but the general effect still remains Honours students may look at the book for background material, chemists generally for the mathematical treatments of the concentration–time curves of various complex reactions But there is nothing here comparable to the general monographs of, say, Kassel or

Hinshelwood—nothing to inspire the research workers in chemical kinetics, to draw young people into the subject, or to give succour to those peris outside paradise, the workers in heterogeneous catalysis This is, I fear, a rather dull book a Moskvich rather than a Lotus Elan D D Eley

Turbulent plasmas

Plasma Astrophysics By S A Kaplan and V N Tsytovich Translated by D ter Haar Pp xiii+302 (International Series of Monographs in Natural Philosophy) (Pergamon Oxford and New York, November 1973) £9 50

This book follows on naturally from *An Introduction to the Theory of Turbulent Plasmas* by Tsytovich, bringing together references to many of the papers published by Russian workers in the last few years In this work the authors are concerned primarily with radiation and particle acceleration by turbulent plasmas in the sun, pulsars, nuclei of galaxies, radio galaxies and quasars The authors restrict their attention to weak turbulence where the energy density of plasma waves is much less than the plasma thermal energy density They assert that strong turbulence decays on a time scale which is short compared with astrophysical times leaving the plasma in a state of weak turbulence Recently discovered 'spicons' in laboratory plasmas with wave energy densities large in comparison with the thermal energy density may eventually cast some doubt on the universal applicability of the weak turbulence assumption to astrophysical plasmas Within the weak turbulence assumption the authors discuss three types of turbulence 'heating turbulence', where the energy of plasma waves is dissipated through electron–ion collisions and Landau damping, 'acceleration plasma turbulence', where the wave energy is lost in accelerating particles, and 'radiation plasma turbulence' where low frequency plasma wave energy is lost by the generation of high frequency electromagnetic waves produced by the interaction of plasma with particles

The book is divided into four chapters· the first reviews, with liberal reference to the literature, laboratory and theoretical developments in plasma turbulence The second chapter deals with sporadic solar emission, for example, how electromagnetic waves may be generated by ion-sound turbulence The third discusses how turbulent processes can lead to observable radiation in for example radio galaxies and quasars, and the book ends with a discussion of pulsar emission

The authors consider that the most important causes of plasma turbulence, at least in a narrow frequency range are beam, ion drift, loss-cone and anisotropy plasma instabilities To these one should probably now add parametric instabilities occurring in the wave zones of pulsars It is not yet clear whether refractive index effects, incurred by requiring a dense plasma to act as a vehicle for the turbulence, will inhibit radiative processes involving relativistic particles The turbulent reactor production of a cosmic-ray ion power low spectrum meets with some difficulty if located in pulsars The authors prefer to have the source in the nucleus of the galaxy, the implication for isotropy is not clear from their arguments

The book is clearly written and the translator is obviously familiar with the scientific content of the work The book is a very important addition to the astrophysicist's library, the new graduate student might however consider reading a more elementary text before attempting it P Stewart

Classifying vegetation

Ordination and Classification of Communities Edited by R H Whittaker. Pp x+738 (Handbook of Vegetation Science, Vol 5) (Junk The Hague, 1973) Dfl 160

A decade ago the production of a volume such as this would not have been possible, but through the agency of the International Society for Plant Geography and Ecology symposia, the different approaches to vegetation, each with its own recondite peculiarities have been interchanged, discussed and dissected But this is only half the story To coax and cajole nineteen eminent practitioners from eight countries to contribute to the tome is no mean feat, and the praise for the commonplace drudgery and exasperating mechanics of coordination of the contributions falls firmly upon the energetic frame of Robert Whittaker There can be no doubt that the dynamic personality of this man and the depth of scientific respect which he commands made the project a viable one, and the patient understanding provided by the publishers helped to ensure a production of the highest quality

The book is divided naturally into three sections—direct gradient analysis, indirect gradient analysis and classification The first section contains five papers, four of which have Whittaker as senior author, and a fifth written by Sobolev and Utekhin of the Soviet Academy of Sciences, Moscow Much of Whittaker's contribution seems to be a new presentation of his earlier works, particularly his 1967 *Biological Review* paper, but as a part of a general overview of methodology, its presence is vital The two Russian authors provide a refreshing interpretation of the much abused Ramensky continuum concept which makes possible a far deeper insight into this approach than has been previously possible

The second section contains typically thorough and informative papers on sample similarity and species correlation by Goodall, factor analysis by Dagnelie and ordination by resemblance matrices contributed by Orloci The methods of comparative ordination evolved by the Wisconsin school some twenty years ago are re-examined by Cottam, Goff and Whittaker and, like the paper on matrix and plexus techniques by McIntosh will win many plaudits for its simplicity and clarity of presentation But pride of place in this section must be given to the invaluable paper of Whittaker and Gauch which provides a realistic and erudite evaluation of ordination techniques using such criteria as efficiency, relative freedom from distortion of sample relationship and computational expenditure

Whittaker is also responsible for two chapters in section 3—one, his familiar enumeration of the bases of vegetation classification and the different traditions which have evolved, the other a short discussion on the nature of dominance types which neatly complements the paper on the physiognomic approach by Beard To many European phytosociologists, chapters 15 to 18 and 20 will prove to be invaluable, if not the highlight of the book They comprise papers written by practitioners from various European countries who are fully conversant with the intricacies of and interpretations to be derived from methods which they themselves have used extensively In this respect, the contributions of Frey, Alexsandrova and Trass and Malmer on the Finnish, Russian and Scandinavian/Baltic approaches respectively help to fill a comparatively large gap in readily available literature in the English language The synusial approach to classification is admirably dealt with by Barkman who covers a wide range of applications from epiphyte societies to aquatic vegetation types In their exposition of the Braun–Blanquet methods, Westhoff and van der Maarel provide what is perhaps the best insight into the methodology in all its facets that has yet been written in English A contribution on numerical taxonomy from Goodall completes this comprehensive section and the book

There can be no doubt that this work will become one of the ecological classics It is already my *vade mecum vegetatio* D W Shimwell

obituary

J. Bronowski

Dr Jacob Bronowski died on August 21, 1974, aged 66 A mathematician, he won acclaim for his work in popularising science in books, on radio and in television broadcasts

Bronowski was born in Poland and came to Britain in 1920 He read mathematics at the University of Cambridge from 1927 to 1930 and continued research there until 1933 A senior lecturer at the University of Hull from 1934 to 1942 he left to head a number of statistical units dealing with the effects of bombing In 1945 he wrote the classic report *The effects of the Atomic Bombs at Hiroshima and Nagasaki*

He was engaged in applying mathematical analysis to the economics of industry until 1950 when he became Director of the Coal Research Establishment of the National Coal Board and was responsible for the development of a new process for making smokeless fuel In 1964 he joined the Salk Institute for Biological Studies in San Diego, becoming Director of the Council for Biology in Human Affairs in 1970

Bronowski published many books including *Science and Human Values*, and *The Western Intellectual Tradition* He was a popular broadcaster and his most recent television series was the award winning *The Ascent of Man* made for the BBC

L. C. Craig

Lyman C Craig whose invention of the counter-current distribution process has been particularly useful for the isolation and study of synthetic antimalarials, antibiotics, hormones and proteins died on July 7, 1974

Dr Craig began his structural studies on the alkaloids of ergot in 1933, when he came to The Rockefeller University as an assistant to Dr W A Jacob, and later isolated lysergic acid During the Second World War he worked on the chemistry of antimalarial drugs for the Office of Scientific Research and Development

R. Cruickshank

Professor Robert Cruickshank, CBE, died on August 16, 1974 aged 74 He was prominent in forging many links between academic and public health microbiology

He graduated from the University of Aberdeen in 1922 and, in 1928, was appointed Lecturer in Bacteriology at the University of Glasgow and Bacteriologist to Glasgow Royal Infirmary Eight years later he became one of the first group pathologists of the London County Council and, in 1945, was appointed Director of the Central Public Health Laboratory Service He succeeded Alexander Fleming as Professor of Bacteriology at St Mary's Hospital in 1948 and became Director of the Wright Fleming Institute of Microbiology From 1958 until his retirement in 1966 he was Professor of Bacteriology at the University of Edinburgh

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Announcements

Appointment

G. R. Serjeant has been appointed director of the **Medical Research Council's Epidemiology Unit** in Jamaica

International meetings

September 23–27, **22nd Symposium of Vertebrate Palaeontology and Comparative Anatomy,** Manchester (D W Yalden, Organiser, 22nd Symposium of Vertebrate Palaeontology and Comparative Anatomy, Department of Zoology, The University, Manchester M13 9PL, UK)

October 2–5, **International Conference on Surgery, Traumatology, Angiology Anaesthesiology and Reanimation,** Budapest (Office for Conference Organisation, (MOTESZ) H-1361 Budapest, POB 32, Hungary)

October 3–6, **6th International Symposium on Clinical Pharmacology and Annual Meeting of the International Society of Clinical Pharmacology,** Regensburg (International Symposia on Clinical Pharmacology, General Office, D-8400 Regensburg 1, PO Box 345, German Federal Republic)

October 7–12, **International Exhibition and Symposium on Elements and Systems for the Preparation, Collection and Transmission of Data and other Peripheral Units of Computers: DATOS 74,** Bratislava (House of Technology of the Slovak Scientific and Technological Society, Bratislava, Kocelova 17, Czechoslovakia)

October 14–16, **2nd International Symposium on Oncogenesis and Herpes-Virus,** Erlangen (Dr H zur Hausen, Institut fur Klinische Virologie, Erlangen, Germany)

October 16–18, **6th Meeting of the International Society of Paediatric Oncology,** Genoa (Sixth Meeting of the International Society of Paediatric Oncology, Department of Paediatric Haematology and Oncology, Inst Giannina Gaslini, via 5 Maggio 39, 16418 Genova-Quarto, Italy)

October 21–25, **International Conference on the Properties of Hydrazine and its Potential Applications as an Energy Source,** Poitiers (Centre National d'Etudes Spatiales (CNES), Department des Affaires Universitaires, 129 rue de l'Universite, 75327 Paris Cedex 07, France)

October 27–November 1, **8th Pan American Congress of Endocrinology,** Buenos Aires (Secretariat, CENI S A, Avda Rogue Saenz Pena 1110, Casillo de Correro 2539, Buenos Aires, Argentina)

October 28–November 2, **International Conference on Plasma Theory,** Kiev (Professor Sitenko, Institute of Theoretical Physics, Metrologicheskaya St, 14-Kiev, USSR)

November 4–7, **5th European Meeting of Radioisotope Producers,** Athens (P Papadimitropoulos, Director External Relations Divisions, Greek Atomic Energy Commission, Nuclear Research Center, 'Demokritos', Aghia Paraskevi Attikis, Athens, Greece)

November 4–8, **3rd International Symposium on Critical Care,** Rio de Janeiro (Dr Brenildo Tavares, Director, International Symposia on Critical Care, C Postal 14700, ZC-95, Rio de Janeiro, Guanabara, Brazil) ●

November 4–13, **9th International Scientific Congress on the Cultivation of Edible Fungi (Part 1),** Tokyo (9th International Scientific Congress on the Cultivation of Edible Fungi, Dr K Ono, c/o Secretariat, RN606, Second Mitsui Building, 12-chome Muromachi, Nihonbashi, Chuo-ku, Tokyo, Japan)

November 5–6, **London Symposium on Radioimmunoassay,** London (Scientific Symposia Ltd, 121 King Street, London W6 9JG)

November 6, **Electron Beam Effects at Surfaces,** London (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX)

November 9–11, **5th Symposium on Plasma Physics and Controlled Nuclear Fusion Research,** Tokyo (International Atomic Energy Agency, P O Box 590, A-1011, Vienna, Austria)

November 15–18, **9th International Scientific Congress on the Cultivation of Edible Fungi (Part 2),** Taipeh (Dr Ren-jong Chiu, JCRR, 37 Nan Hai Road, Taipeh, Taiwan)

November 20–25, **Symposium on Foetal Antigen Expression in Cancer,** Isawa, Japan (Dr E Alpert, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts, USA *or* Dr H Hirai, International Research Group for Carcino-embryonic Antigens, Hokkaido University Medical School, Sapporo, Hokkaido, Japan)

November 21–22, **Symposium on Nutrition and Aging,** New York (Director, Institute of Human Nutrition, Columbia University, 511 West 166th Street, New York, N Y 10032)

November 26–28, **Conference on the High Temperature Reactor and Process Applications,** London (British Nuclear Energy Society, Institution of Civil Engineers, 1–7 Great George Street, London SW1P 3AA)

Erratum

In the article "Structure of yeast phenylalanine tRNA at 3 Å resolution" by J D Robertus *et al* (*Nature*, **250**, 546, 1974) the last sentence of the legend to Fig 6 should read 'The region marked f is explained in the text as region 6'

Reports and Publications

Great Britain

Fourth Annual Report of the Advisory Committee on Oil Pollution of the Sea Research Unit on the Rehabilitation of Oiled Seabirds Pp 27 (Newcastle-upon-Tyne Department of Zoology, The University, 1974) [17

Public Health Laboratory Service Monograph Series No 4 The Distribution and Identification of Non-Fermenting Bacteria By J J S Snell Pp vii + 45 (London HMSO, 1973) 50p net [37

Does Research threaten Privacy or Does Privacy threaten Research?—Report of a Study Group (Publication No 74/1) Pp 23 (London British Association for the Advancement of Science, 1974) 50p (40p to members) [37

Enquiry 1974 10th Anniversary Edition (The Science Journal of Harrow County School for Boys) Pp 112 (Harrow Sales Manager, *Enquiry*, The County School for Boys, 1974) 40p + 17p postage [47

Cancer Research Campaign 51st Annual Report 1073, Incorporation Research Supported During 1974 Pp 157 (London Cancer Research Campaign, 2 Carlton House Terrace, 1974) [47

Scientific Proceedings of the Royal Dublin Society Series A Vol 5, No 6 Contributions to the International Geodynamics Project Pp 87-122 + plates 11-14 £1 50 Vol 5, No 8 The Occurrence of Cordierite and Staurolite in a Part of the Leinster Granite Aureole a Textural Study By P S Kennan Pp 131-136 30p Vol 5, No 9 The Graiguenamanagh Belt in the Leinster Granite By J C Brindley Pp 137-144 30p Vol 5, No 10 The Variable Granites at the Northern End of the Leinster Pluton By J C Brindley and L N Gupta Pp 145-158 + plate 16 40p Series B Vol 3, No 17 A Survey of Drinking Waters of Northern Ireland with particular reference to Their Fluoride Content By C McKay Pp 221-249 £1 (Dublin Royal Dublin Society, 1974) [47

Mathilda and Terence Kennedy Institute of Rheumatology 7th Annual Report 1973 Pp 80 (Hammersmith, London W6 The Mathilda and Terence Kennedy Institute of Rheumatology, 1974) [87

Philosophical Transactions of the Royal Society of London A Mathematical and Physical Sciences Vol 277, No 1262 Seismicity and Structure of the Zagros (Iran)—the Main Recent Fault Between 33° and 35°N By J S Tchalenko and J Braud Pp 1–25 + plates 1-3 (London The Royal Society, 1974) [87

Research Grants in the Natural Environmental Sciences NERC Research Grant Support Current Between 1 June 1972 and 31 May 1973 Pp v + 102 (London Natural Environment Research Council 1974) [87

Scottish Plant Breeding Station Fifty-third Annual Report, 1973/74 Pp 84 (Pentlandfield, Roslin, Midlothian Scottish Plant Breeding Station, 1974) [97

National Radiological Protection Board NRPB-R20 The Risk of Death from Radiation-Induced Cancer as Estimated from the Published Data on the Japanese Atomic Bomb Survivors By S G Goss Pp 26 NRPB-R21 Assessment of Contamination from the Release of Activated Sulphur Hexafluoride from a Q-Tube Neutron Generator Used for Radiotherapy By R P Rowlands and D L O Humphries Pp 10 (Harwell, Didcot National Radiological Protection Board, 1974) [97

Advisory Committee on Oil Pollution of the Sea Annual Report for 1973 Pp 28 (London Advisory Committee on Oil Pollution of the Sea, 39 Victoria Street, 1974) [107

Other Countries

Final Report, Conference of Ministers of African Member States Responsible for the Application of Science and Technology to Development, organised by Unesco with the co-operation of the Economic Commission for Africa and the Organisation of African Unity Dakar, 21-30 January 1974 Pp 68 (Paris Unesco, 1974) [216

Records of the Australian Museum, Vol 29, No 2 The Garfishes (Hemiramphidae) of Australia and New Zealand By Bruce B Collette Pp 11-105 (Sydney The Australian Museum, 1974) $4 [256

Democratic Republic of the Sudan Ministry of Agriculture—Agricultural Research Corporation Annual Report of the Gezira Research Station and Substations, 1968/1969 Pp xi + 338 (Wad Medani Gezira Research Station, 1974) [256

Smithsonian Contributions to the Earth Sciences, No 12 An Atlas of Volcanic Ash By Grant Heiken Pp iv + 101 (33 plates) $2 Smithsonian Contributions to Zoology No 156 A Revision of North American *Capitophorus* Van der Goot and *Pleotrivhophorus* Borner (Homoptera Aphididae) By L A Corpuz-Raros and Edwin F Cook Pp iv + 143 $2 25 (Washington, DC Smithsonian Institution Press, 1974 For sale by US Government Printing Office) [256

CERN—European Organization for Nuclear Research CERN 74-10 On Preconditioning and Convergence Acceleration in Sparse Matrix Problems By O Axelsson Pp v + 21 CERN 74-12 A General-Purpose Amplifier and Read-Out System for Multiwire Proportional Chambers By J Lindsay, Cl Millerin J Cl Tarle, H Verweij and H Wendler Pp 11 (Geneva CERN, 1974) [256

Office de la Recherche Scientifique et Technique Outre-Mer, Bondy et Paris Collection Traviax et Documents ORSTOM, No 26 Étude des Mécanismes Physiologiques de la Formation des Sclérotes chez le *Corticium rolfsii* (Sacc) Curzi Par M Coujon Pp 107 40 francs Travaux et Documents ORSTOM No 33 Le Bassni d'Ambalavao Influence Urbaine et Evolution des Campagnes (sud Betsileo Madagascar) Par M Portais Pp 172 + 8 planches 66 Francs Collection Initiations—Documentations Techniques No 23 Initiation a la Morphologie, La Systématique et la Biologie des Insectes Par M Roth Pp 213 + 44 planches 38 francs Mémoires ORSTOM No 69 Contribution a la Connaissance Physiologique de l'Homme noir dans Milieu Ecologique Etude de Composants Seriques Protides, Fer, Cuivre, Chez l'Africain du Cameroun Par C Cavelier Pp 144 60 francs Memoires ORSTOM No 71 Les Euphausiacés du Pacifique Equatorial et Sue Tropical—Zoogéographie, Ecologie, Biologie et Situation Trophoque Par C Roger Pp xx + 265 100 francs (Paris et Bondy ORSTOM, 1974) [256

US Department of the Interior Geological Survey Professional Paper 731 Geology of the Pulga and Bucks Lake Quadrangles, Butte and Plumas Counties, California By Anna Hietanen Pp v + 66 + 3 plates, $3 45 Professional Paper 768 Lithostratigraphy and Depositional Environments of the Lexington Limestone (Ordovician) of Central Kentucky By Earle R Cressman Pp iv + 61 + 11 plates $8 70 (Washington, DC Government Printing Office, 1973) [266

Smithsonian Contributions to Zoology, No 161 Five New Bomolochid Copepods Parasitic on Indo-Pacific Clupeid Fishes By Roger F Cressey and Hillary Boyle Pp 25 (Washington, DC Smithsonian Institution Press, 1973 For sale by US Government Printing Office) 60 cents [266

CERN—European Organization for Nuclear Research CERN 74-13 Dipole Septum Magnets By R L Keizer Pp 28 (Geneva CERN, 1974) [266

Indian Forest Bulletin No 267 (NS) (Forest Pathology) Microbial Degradation of Pulpwood By B K Bakshi and Sujan Singh Pp 17 (Delhi Manager of Publications, 1973) RS 1, 12p, 36 US cents [266

Forest Research in India 1961-62, 1962-63, & 1963-64, Part 2 Reports from Indian States Compiled by K B L Mathur Rs 5, 58p, US$1 80 1964-65 Pp 59 Rs 1 85, 22p 67 US cents (Dehli Manager of Publications, 1972 and 1973) [266

Australia Commonwealth Scientific and Industrial Research Organization Annual Report of the Division of Fisheries and Oceanography, 1972/1973 Pp 67 (Cronulla, NSW CSIRO, 1974) [276

Smithsonian Contributions to Zoology, No 168 Ecology of the Squash and Gourd Bee, *Peponapis pruinosa*, on Cultivated Cucurbits in California (Hymenoptera Apoidea) By Paul D Hurd, Jr, E Gordon Linsley and A E Michelbacher Pp iii + 17 (Washington, DC Smithsonian Institution Press, 1974 For sale by US Government Printing Office) 60 cents [276

Royal Ontario Museum Life Sciences Occasional Papers, No 25 A New Subspecies of the Bat *Eumops auripendulus* (Chiroptera Molossidae), from Argentina and Eastern Brazil By Judy L Eger Pp 8 (Toronto Royal Ontario Museum, 1974) [286

Smithsonian Contributions to Palaeobiology, No 15 Permian Brachiopods of West Texas, II By G Arthur Cooper and Richard E Grant Pp vii + 233-793 (plates 24-191) (Washington, DC Smithsonian Institution Press, 1974 For sale by US Government Printing Office) $9 65 [286

Annual Summary of Information on Natural Disasters Earthquakes, Tsunamis, Volcanic Eruptions, Landslides, Avalanches, No 7, 1972 Pp 106 (Paris, The Unesco Press, 1974) [17

Bulletin of the Fisheries Research Board of Canada, No 185 Benthic Errantiate Polychaetes of British Columbia and Washington By Karl Banse and Katharine D Hobson Pp x + 112 (Ottawa Information Canada, 1974) $4 50 [17

The Enterprise, Wisconsin, Radiation Forest Preirradiation Ecological Studies Edited by Thomas D Rudolph (TID-26113) Pp v + 150 (Oak Ridge, Tennessee US Atomic Energy Commission, Technical Information Center, 1974 Available from National Technical Information Service, US Department of Commerce, Springfield, Virginia 22151, USA) $7 60 [17

Reports of the Finnish Geodetic Institute No 74-2 Time Keeping Methods Applied in Finland By K Kalliomaki and J Kakkuri Pp 37 (Helniski Finnish Geodetic Institute, 1974) [17

Anticancer Agents Recently Developed in the People's Republic of China—a Review By C P Li (A Publication of the Geographic Health Studies, John E Fogarty International Center for Advanced Study in the Health Sciences) (US Department of Health, Education and Welfare Publication No (NIH)74-441) Pp x + 255 (Washington, DC Government Printing Office, 1974) $2 [17

A New Look at Supplements Casting Off Old Prejudices (Based on a presentation of safety and efficacy submitted to the Food and Drug Administration OTC Review Panel on Vitamins, Minerals and Hematinics) Pp 14 (Washington, DC Council for Responsible Nutrition, 1776 K Street NW, 1974) [17

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Low Level Lead Toxicity and the Environmental Impact of Cadmium (*Environmental Health Perspectives* Experimental Issue No 7, May 1974) Pp iv + 323, (National Institute of Environmental Health Sciences, National Institutes of Health, Education and Welfare) (Washington, DC US Government Printing Office, 1974) $3 05 [27

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nature

Volume 251 September 20, 1974

Right and wrong ways of expressing concern

We publish a letter from Professor F. Janouch on page 181 which together with the appeal from Professor B. Peleska to the World Federation of Scientific Workers (WFSW) (*Nature*, July 12) makes for most disturbing reading.

For a long time there has been talk of the miseries of some members of the academic profession in Czechoslovakia whose political views did not accord with those of the government. Much of this talk, however, has been coupled with a fear that to name names in the West would simply lead to greater personal distress in the East. And some of the talk has been simply of a subjective nature from visitors—that Prague has become 'the most miserable of capitals' and so on. Fear of naming names is not ill-founded; Professor Peleska has already, it is said, been visited by the police in connection with the letter sent to the WFSW and reprinted with his permission in *Nature*.

Czechoslovakia then, according to many with whom we have spoken, is at present about as bad a place as any to be an academic devoted to the spirit of liberal enquiry and freedom from repression. What can academics do in a public sense about preventing a breakdown in the standards which they regard as proper for the pursuit of learning? There are, we believe, no essential differences in approach between concern for Czechoslovakia and concern for, say, Chile.

It is easier, of course, to spell out what they shouldn't do.

- They shouldn't assume that an intellectually distinguished body is capable of leading the way diplomatically. The Royal Society in Britain, for instance, has been under much criticism in recent years for its failure to come out publicly on such issues as Soviet treatment of certain scientists. The standard defence is that much good work is done behind the scenes in person-to-person meetings with opposite numbers. This may well be true, but surely the real answer is that a society founded on intellectual distinction should not be asked to do a job requiring a high degree of political acumen.
- They shouldn't expect a politically committed body to deliver the goods. The WFSW has done some excellent bridge-building in the past and may appear, through its clear links with Eastern Europe, to be just the right vehicle for transmitting criticism. But the links with the East have been at the expense of strength in the West and particularly in the United States where an enormous number of scientific workers are and yet where few have ven heard of the federation. It is sad that Professor Burhop, president of the federation, should have chosen o respond to the appearance of the Peleska letter by counter-examples of western victimisation, but it shows e weakness of any organisation which as the Roses put it in *Science and Society* is a victim of cold-war and East–West polarisation.
- They shouldn't confine their concern to th specialisation. An academic community divi science, sociology and the arts or whatever is w may be to the point of ineffectualness. The learning must be indivisible on such matters.

But what positive steps are there to recomm often academics have used the wrong channels protest? Two seem to be under-explored and wo more attention—the approach to politicians and th of the press.

The elected politician with either his access to, or desire to pose problems for, a government has bee underestimated as a means of applying pressure in shaping foreign policy. And the ability of foreign policy to include specific references to the problems of oppressed groups has been inadequately used by academics—although a start has recently been made in the inclusion of concern for Soviet Jews in US foreign policy. If a hundred professors in any one country took individual initiatives to harangue their representatives on a matter so obviously not involving self-interest, could that country's foreign policy remain unaltered?

If approaches to politicians might help on a national front, approaches to the journals could rapidly move information around the world in order to create the necessary international climate. Who can doubt that wide publicity of the preposterous behaviour of the Soviet authorities in excising names that displease them from published papers serves a valuable purpose. And the scientist in particular, wedded to the importance of documentary evidence is at once more able to appreciate hard fact and more likely to be able to supply it. It is all-but-impossible for an editor to resist a well-documented story.

A hundred years ago

The will of the late Girolamo Ponti, of Milan, which has just been published in the *London Gazette* by order of Lord Derby, is likely to give rise to some trouble before it can be carried into effect. The testator has bequeathed a considerable portion of his property to the "Academies of Science of London, Paris, and Vienna," to be divided among them in equal proportions, for the purpose in each case of founding, with the proceeds resulting from investment, two competitions yearly on the subjects of Mechanics, Agriculture, Physics and Chemistry, Travels by Sea and Land, and Literature. The committees to be appointed by the societies are instructed to give preference to those competitors who will have advanced any of the subjects mentioned by original discovery. The relatives of Signor Ponti are to dispute the will, and those London societies that think they have claims upon the legacy are urged to bring them forward at once. There can be no doubt which societies are meant in the case of Paris and Vienna; and at first sight there appears to be little doubt as to what body the title of "Academy of Science of London" would most appropriately apply.

From *Nature*, **10**, 427, Sept. 24, 1874.

Strategy for a hungry world

Dr D. S. Miller of Queen Elizabeth College, and Dr P. R. Payne of the London School of Hygiene and Tropical Medicine take issue with a recent article in Nature *on the value of amino acid supplementation, especially in developing countries.*

s been claimed on a number of ions during the past few years it is possible to bring about sub- tial improvements in the health well being of the poor people of world by the application of some atively cheap and simple technology. ich a possibility seems theoretically oubtful and we feel that it is most mportant that attention should not be distracted from the real problems of social and economic developments by exaggerated claims for the impact of some particular technique. It is for this reason that Aaron Altschul's restatement of the apparent benefits of fortification of foods with amino acids[1] needs a critical appraisal.

We might begin by pointing out the significant fact that although the advantages of adding amino acids to human foods have been vigorously promoted for at least 20 years, and the technology for doing so has been available for as long, there is to date not a single example of a controlled field trial on a population basis which has shown any measurable benefit to man. We believe there are both nutritional and logistic reasons for this.

The nutritional basis for amino acid fortification rests upon the proposition that a specific deficiency of protein is a common cause of ill health. Such a statement requires definition. It is important to distinguish between the physiological needs for protein as a nutrient and the desire for foods such as meat, milk, eggs and fish. It has been a consistent feature of the arguments of those who advocate protein or amino acid fortification as a cure for malnutrition that they confuse the problem of meeting physiological needs for protein with the problem of meeting demands for aesthetically satisfying diets. Perhaps it is not surprising that many people can see no way in which the demands of the rich for meat could (or even should) be moderated in the light of the needs of the poor for bread. It is surely hypocrisy to pretend on the one hand that these demands and needs are both expressions of the same protein problem, and on the other to suggest that the needs of the poor can be satisfied by fortification of staple foods with a tasteless, odourless powder which will help neither to satisfy hunger or incidentally to improve the nutritive value of the diet.

The second problem of definition is to distinguish between diets which are 'limited' by a particular amino acid and those which are 'deficient'. For example the traditional English breakfast of porridge, bacon and eggs, toast and coffee contains a protein mixture that if fed under carefully controlled conditions could be shown to be limited by methionine. But this is neither evidence that the UK population is at risk from methionine or protein deficiency, nor does it justify the fortification of breakfast foods with amino acids. A diet may only be considered deficient in an amino acid if it contains less than the requirements of the consumer.

Dr Altschul correctly points out that standards of protein requirements are now "lower than before". What he does not point out is that one takes the latest FAO/WHO standards[2], it can be shown that the required percentage of calories from protein in mixed diets as eaten by man is only 8%. This assumes that the efficiency of protein utilisation of human diets eaten in poor countries is about 65%. In fact, the calculated efficiency derived from the methods given by the FAO/WHO report is higher than this at about 90% which would lower the required percentage of dietary protein even more. Thus the cited projection that the protein content of the diets eaten in South Asia will fall from 10.2% to 9.8% by 1980 does not indicate impending disaster. Of course there are those who argue that the poorest people are eating bread or rice alone. These cereals will have an efficiency of utilisation about 50%, and hence the required protein content of the diet would rise to 10%, still covered by cereals alone. Another argument is that cereals do not contain enough protein for the vulnerable groups in the population such as preschool children. But the latest requirements indicate that they also require only 10% cereal protein as a percentage of the calories. Thus simple protein deficiency cannot occur in areas where cereals are the cheapest food, and amino acid supplementation would therefore be a wasted effort. Simple protein deficiency can only occur where the staple food contains less than 10% protein such as cassava, sago, and plantain. Since these foods contain virtually no protein, supplementation with a few amino acids cannot increase their useful protein content by changing protein quality.

This is not to say that there is no such thing as protein-calorie malnutrition. This syndrome arises chiefly because children do not eat enough food. It is complicated by disease, especially diarrhoea, respiratory infections and measles, but the prime cause is insufficient food. We were interested to read in the same issue of *Nature*, the account by Ethiopian relief workers and their critical assessment of protein-rich foods. Indeed your editorial correctly praised this group for their design of an energy-rich food for use in such situations. It is clearly non-

sense to supply developing countries with single nutrients, such as amino acids or vitamins, when some people are unable to buy and consume enough of the traditional diet which has sustained the population for centuries

Turning now to the logistic problems of amino acid fortification, we would suggest the following conditions before any project should be implemented

- That the limiting amino acid in the diet as distinct from the staple food, must be known The extent of the limitation must also be known In practice, cereal-based diets as eaten are found not to be limited by lysine Miller and Donoso[3] have shown that the limiting amino acid of most diets, including cereal diets, is methionine Also that the improvement in the effective protein content when methionine is added is trivial in comparison with the data cited by Dr Altschul concerning the effect of the addition of lysine to flour alone
- That the supplemented food is consumed by the vulnerable groups in the population This is normally only practicable where the staple foods are centrally processed and almost impossible where the major proportion of the population are subsistence farmers
- That the project is the cheapest method of improving the diet as a whole Since the chief cause of malnutrition is poverty, amino acid supplementation should be regarded by governments as an alternative to raising the standard of living Thus to compare a scheme for adding lysine to wheat with one for improving milk production from marginal grassland, purely on the basis of the yield of protein is misleading Improving agriculture provides employment, can be achieved within the country, and produces a product which is both aesthetically satisfying and acceptable Finally we must point out that Dr Altschul does not fully quote one of his references[4], which indicates that the cost of fortification of all cereals in India would be no less than 16% of the Central Government Budget, or approximately five times the money spent on health

We conclude that amino-acid fortification of staple foods should be rejected on nutritional and logistic grounds The best solution to the problem of malnutrition is to raise the standard of living and to improve traditional agriculture Aid to the subsistence farmers is the best way to achieve both

[1] Altshul, A M, *Nature* **248**, 643 (1974)
[2] *FAO/WHO Technical Report No 522* (1973)
[3] Miller, D S, and Donoso, G, *J Sci Fd Agric*, **14**, 345 (1963)
[4] Schertz, L P, *Nutr Rev*, **31**, 293 (1973)

international news

Doubts over US in India

from Narender K Sehgal

THE World Health Organisation (WHO) has been carrying out allegedly secret research projects in India, financed by agencies of the U S government It has recently come to light that the U S Army has been an interested recipient of the research results from these projects

The WHO experiments are being conducted (some have been completed) at the Genetic Control of Mosquito Unit (GCMU) in New Delhi, Bombay Natural History Society (BNHS) and the malaria eradication station in Jodhpur (Rajasthan state) The projects in question relate to the genetic control of mosquitoes, bird migration and the like—seemingly scientific investigations, till one takes a closer look at the related details But Indian experts feel that the experiments could also provide data on the availability, behaviour and peculiarities of disease carriers in India—information that could be of value in biological or germ warfare

Until recently the GCMU had been collecting data on the ecology, behaviour and dispersal patterns of mosquitoes in South Delhi and in Faridabad, a sprawling industrial complex near the capital Some scientists have expressed fear that, using these data, an enemy could conceivably employ mosquitoes for the effective transmission of germs and viruses Also, the GCMU conducts most of its experiments in and around Delhi—an area known not to be endemic for malaria or filariasis—instead of in the really endemic areas around the country

The GCMU proposes to launch a big experiment early next year in Sonepat (again near Delhi) aimed at genetic control of *aedes aegypti*—in spite of the fact that the GCMU's first priorities ought to be malaria and filariasis carriers Malaria is back in India and filariasis is endemic in an area occupied by a fourth of the Indian population Dengue, unknown in India before July 1963 when it was detected for the first time in Calcutta, appears sporadically Yellow fever is nonexistent in India, not a single case having been reported in ages This, it is argued, may be because of some 'balancing virus' that ensures protection against this disease But the GCMU's genetic experiments could reduce or destroy this natural 'balance' and render the country vulnerable to yellow fever

Indian scientists taking part in the GCMU's projects point out that their project leaders are, and have been in the past, U S government scientists Two years ago, the GCMU attracted public attention when it was found to have been polluting village wells—apparently in the course of scientific experiments—with chemicals suspected to be cancer-causing and prohibited in the USA Since then secrecy around GCMU projects has been reportedly tightened

Another controversy that has been raging among the scientific community here has to do with the role of Indian scientists and research organisations in programmes sponsored jointly by the Migratory Animal Pathological Survey (MAPS) of the U S Armed Forces Institute of Pathology and WHO Reacting to criticism in the Indian Parliament the president of the Bombay Natural History Society, in a letter to a New Delhi daily, stoutly defended his society's collaboration with the MAPS on bird migration projects, he denied allegations of secrecy, ongoing collaboration arrangements with the MAPS and employment of U S personnel in bird migration studies

Following this a scientist of the Jawaharlal Nehru University in New Delhi, in a letter to the same daily, pointed out a few things the BNHS president forgot to mention in his letter Namely that (i) the blood samples of the birds caught by the BNHS were not analysed in India but were sent for the purpose to the Smithsonian Institute and the MAPS in the USA, (ii) two American scientists belonging to the Smithsonian were associated with the BNHS-MAPS programmes in India, (iii) the services of the BNHS and VRC (Virus Research

Centre of Poona) were made available to the U S Army agencies at nominal cost, (iv) the knowledge gained by the Indian scientists at BNHS and VRC was only partial, and probably the least significant if it did not include the details of the blood sample analyses and the overall data on bird movements and induced infections, and that (v) only a coordinating agency outside this country, which obtained comprehensive data from other units, could assess the military uses or significance of such experiments

There is no doubt that the WHO projects in India are carried out with the approval of the government of India through its ministry of health, but when the matter of involvement of the U S Army in some projects was raised in the Rajya Sabha (upper house of the Parliament) the government merely assured agitated members that it would see to it that national interests were not endangered by these projects This routine assurance to Parliament members has not impressed many in the scientific community, and demands are being made for a thorough probe into the whole situation □

Science policy in the Netherlands

from Arie de Kool, Rotterdam

DISCUSSIONS on science policy are running so high in the Netherlands that even scientists are beginning to take an interest The radical Bond van wetenschappelijke arbeiders (Union of Scientific Labourers) has been publishing reports on science policy ever since it was established about six years ago, and recently they have begun to cooperate closely with the Pugwash-based Verbond van wetenschappelijke onderzoekers (Union of Researchers) Together, the two unions have brought out a joint proposal for a democratic structure for science policy

Their proposed structure is supposed to keep technocracy out, and guarantee a maximum amount of freedom and power over his own work for the research worker while at the same time safeguarding scientific standards It is based on university organisation where one finds at the lowest level a section (for example, the solid state physics section or the French section), governed by a council on which scientific staff, technical assistants and students are represented (but in such a way that students can never obtain a majority) Several sections form a faculty, with its own council

According to the new proposal the sections would not only join within the university, but all sections for, say, solid state physics, would elect a "working community" at a national level This working community would advise upward on the urgency of different programmes, and also receive money to pass out to the working groups in the universities In fact working communities are now functioning rather well on the average, doing exactly this There would be two differences, they would be elected by the scientists involved (and not appointed by the Dutch version of the National Science Foundation, ZWO, or one of its subsidiaries), and they would be responsible for about one third of the money going to a specific discipline

Over and above the councils, we are to find a national council for higher education and scientific research This is an almost purely political body, to advise government and parliament on matters contained in the name, and at the same time an executive body, handling all the money One third of the research money would go to the universities to be spent at will (as long as it is for research, the democratic structure of the university is supposed to guarantee that this money is well spent) About one third goes to the universities, but would have to be spent in the faculties more or less proportionally to the number of students And one third would go to the discipline councils to hand further down

The idea behind this being that one needs a certain degree of centralisation if one wants any science policy at all On the other hand, too much of it tends to kill the initiative, and close off new roads and new research, while there may be a strong tendency to overemphasise certain fashionable fields In this structure, one would see about one third handled centrally, about one third at the university level, and about one third (the per-student amount) "at the basis", in the working group itself At any level the researcher is supposed to have a certain degree of influence through his representatives, but of course, this private influence diminishes as one goes up higher in the structure

Industry has been almost left out The report says that since none of the proposed institutions could be expected to effectively exert power over industrial science, it would be wrong to have an industrial influence in the different councils However, a better coordination of industrial and university research in socially relevant fields, is seen as worthwhile In the long run government might even want to stimulate industrial research in those fields, and want to put a brake to other fields Therefore industry should be asked to report on its scientific and technical activities, for instance, to the national council □

Trouble in the air

by Colin Norman, Washington

FOR the past couple of years a number of industrial groups, taking their cue from the automobile industry, have been waging a pitched battle against some of the Federal government's plans for cleaning up air pollution Their prime objective has been to persuade Congress to emasculate some key provisions of the Clean Air Act—the foundation of the government's air pollution strategy—which they consider unduly stringent, scientifically invalid and, of course, inordinately expensive

But the rug was pulled from under industry's feet last week when the National Academy of Sciences unveiled a massive study of the Clean Air Act and its scientific foundation Although the committee which conducted the study posed more questions than it answered, and hedged all its conclusions with a string of qualifications, it said that there is "no substantial basis" for weakening key provisions in the act, and that the costs of cleaning up automobile emissions are at least commensurate with the benefits

A few Congressional staff members centrally involved in the debate over air pollution standards all said in response to inquiries last week that the Academy's study will probably provide Congress with sufficient ammunition to ward off challenges to the Clean Air Act which are certain to arise next year—the act is due for review and possible amendment in 1975

The Clean Air Act sets maximum levels of various pollutants which are allowed in the atmosphere, the chief intent of these so-called air quality standards being to protect public health In addition, the act specifies maximum levels of hydrocarbons and oxides of nitrogen which can be discharged by new automobiles The air quality standards have, however, been attacked for being stricter that necessary to protect public health, and the automobile emission standards have come under fire for being too costly to meet

But the Academy's study, which was conducted under contract to the Senate Public Works Committee, said that the standards have, in general, been supported by the evidence which has accumulated in the three years since they were set Moreover, "the safety factors provided by the air standards are much smaller than is usual in regulating other environmental pollutants such as radioactivity", the study concluded

In fact, using calculations which are admittedly uncertain but which are derived from a variety of sources, the Academy committee reckons that auto-

Washington seen

by Colin Norman

Ever since Gerald Ford was elevated to the Presidency early last month, there has been a good deal of speculation among members of the scientific establishment on whether or not he will resurrect some of the White House science policy machinery which his predecessor abolished. With galloping inflation and Presidential pardons keeping him occupied, Ford has, of course, shown no indication of rushing to deal with the matter, but there are a few suggestions that perhaps he might attend to it at some time.

First, he met earlier this year with a number of scientific society presidents and was reported to be receptive to the need for a scientific presence to be reestablished in the White House. Second, he was personally briefed on the recent report of the National Academy of Sciences which urged him to bring the science policy machinery back in from the National Science Foundation, which is where Nixon dispatched it. He was said to be a willing listener. And third, his nomination of Nelson Rockefeller as Vice President brings to the White House a man who is well known for seeking out science advice, albeit mostly from Edward Teller, the so-called father of the United States H-bomb.

But perhaps the most decisive move could come from Congress. Last month, the Senate Commerce Committee reported out a bill which would set up a Council of Science and Technology Advisers at the President's elbow. The bill has also been referred to the Senate Committee on Aeronautical and Space Sciences, which will probably approve it this week. There is thus a chance that the idea will get the blessing of the entire Senate before it adjourns in mid-October, in which case the whole matter will be brought rather abruptly to Ford's attention.

So far, however, nobody has been able to demonstrate convincingly that the present arrangement is not working properly, or that a White House science policy office would work any better under Ford than it did under Nixon.

● Officials at the National Science Foundation are now trying to decide how to apportion a $20 million budget cut which was recently imposed by Congress, but they have little choice except to take virtually all of it out of basic research.

Congress, demonstrating once again that its rhetoric in support of science and technology doesn't count for much when it actually comes to spending money, cut $20.05 million from the budget request for NSF, but also directed the foundation to spend some $6.8 million more than it wanted on a few politically sensitive education programmes. The upshot is that $26.85 million must be shaved from other NSF activities.

Congress itself dictated that at least $5.5 million must be taken from the programme of Research Applied to National Needs (RANN), and that another $1.4 million should come from a research management programme, but it left NSF officials free to decide where to cut the other $20 million.

According to NSF budgetary officers, however, the only area that can be cut is the foundation's support of basic university science, and they reckon that as much as $18 million could come from there.

In language uncharacteristically blunt for that decorous institution, the National Academy of Sciences has criticised the Environmental Protection Agency's management of its research and development activities, and has suggested that EPA should scrap its present planning and management procedures and devise some new ones.

The criticisms were contained in a report drawn up by an ad-hoc Academy committee under the chairmanship of Dr Robert W. Berliner, former Deputy Director of the National Institutes of Health.

The committee found that planning of EPA's research and development programmes is separated from responsibility for their execution, which has led to "severe resentment" among the agency's scientists. Research priorities do not necessarily reflect the needs of EPA's regulatory officers, the planning system is excessively complex and detailed, there is no long term research programme designed to meet environmental needs, and "relationships between the headquarters and field (staff) are strained at best".

The committee also noted that the fact that EPA's research and development budget has been held constant for the past few years doesn't help matters.

To help remedy the situation, the committee suggests that the present arrangements should be scrapped, that the directors of EPA's laboratories should be given more of a say in the planning process, and that the headquarters staff should be trimmed by decentralising some of the control.

The report is the first to emerge from a $5 million contract between EPA and the Academy under which the Academy will conduct a comprehensive study of EPA's activities. The study was mandated by Congress at the urging of Mississippi Congressman Jamie Whitten, a strong supporter of DDT and other pesticides, who believes that EPA is out to destroy American agriculture.

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mobile pollution could now account for as many as 4,000 deaths and four million days of illness among Americans each year. That represents about one-eighth of the deaths from bronchitis, emphysema and asthma, and one-tenth of the days lost from work each year from respiratory illness.

The committee told Congress that it sees no reason to alter any of the air quality or automobile emission standards, although it does suggest that the standard relating to emission of oxides of nitrogen by automobiles "may be somewhat more stringent than is needed". But the automobile companies will find it difficult even to use that suggestion as ammunition against the Clean Air Act, since the Academy committee says in the next breath that since little is known about interactions between oxides of nitrogen and hydrocarbons in the atmosphere, it would be unwise to relax the standard at this time.

Finally, an amusing footnote to the debate over automobile pollution was provided last week by General Motors. When the exhaust emission standards were first set, the entire automobile industry rose up in arms because the timetable for meeting the standards was so short that they would have to invest huge sums of money in exhaust catalysts—no other technology would have been available in time.

Motor manufacturers argued vehemently that catalysts would be unreliable, that they would decrease engine performance, and that they would greatly increase gasoline consumption. But Congress refused to scrap the timetable and many of the 1975 model cars which still come onto the market this month will be equipped with catalysts. General Motors has consequently changed its tune.

According to a series of full-page newspaper advertisements which the company took out last week: "General Motors believes it has an answer to the automotive air pollution problem . . . and the catalytic converter has enabled GM engineers to improve performance and to increase miles per gallon." It's amazing what a little government pressure can do. ☐

Vancouver boycott supports Plyushch

from Vera Rich

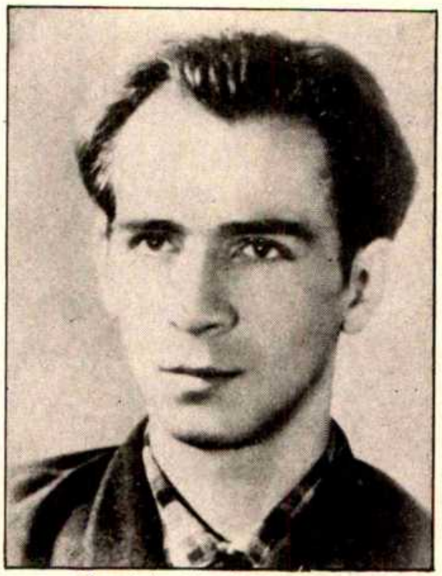
THE right of a scientist to travel abroad for professional purposes is one of the most vexed questions confronting campaigners for human rights in the USSR. Recently the greatest attention has been focused on those Jewish scientists who wish to leave the Soviet Union permanently and settle in Israel—a desire usually denied either on the grounds that the scientist concerned has allegedly had access to classified information, or else that he represents an asset to the State in terms of education and experience. (The fact that scientists who apply for a visa to Israel tend to be fairly rapidly dismissed from their posts does not entirely vitiate the latter argument —the authorities may not wish to make use of the services of these valuable personnel, but they do not wish their services made available elsewhere).

The case of scientists who wish for a temporary visa is far less defensible. These scientists simply wish to travel abroad to a conference, deliver their paper, and return home. There is no question of settling abroad; they wish merely to make a temporary visit. Yet all too frequently, invited scientists fail to arrive at conferences, and their places are filled by uninvited representatives of the Soviet Union, who have little or no no reputation in the field concerned, and who insist on delivering papers which often have only a very peripheral connection with the subject of the conference.

This substitution of delegates can, however, possibly be turned to good account by those who wish to press for academic freedom for their Soviet colleagues. At the recent International Congress of Mathematicians in Vancouver, a number of participants decided to boycott the papers of such uninvited delegates as a protest against the persecution of a number of Soviet mathematicians, notably that of Leonid Plyushch, whose fate was also the subject of an appeal to the Congress by Dr Andrei Sakharov, the founder of the illegal "human rights" movement in the Soviet Union.

Plyushch, a mathematician and cybernetician, was formerly employed at the Cybernetics Institute of the Academy of Sciences of the Ukrainian SSR, specialising in the computer simulation of biological and biochemical processes. In 1968, he was dismissed from his post as the result of a letter which he wrote to the newspaper *Komsomolskaya Pravda*, protesting against the illegality of the trial of the writers Ginsberg and Galanskov. In 1969, he became a founder-member of the illegal "Initiative Group for the Defence of Human Rights", founded by Dr Andrei Sakharov. In 1972, after almost four years without work, and a number of interrogations and harassments, Plyushch was arrested on January 17, 1972, under Article 62 of the Criminal Code of the Ukrainian SSR for 'anti-Soviet activities'. His arrest formed part of a general campaign against the *samizdat* journals *Chronicle of Current Events* and the *Ukrainian Herald*. In January 1973, he was sentenced *in absentia* to indefinite confinement in a special mental institution, suffering, it would appear, from that notable discovery of Drs Lunts, Morozov, *et al.*: "schizophrenia leading to ideas of reform making."

Since then he has been confined in the Dnepropetrovsk "special" psychiatric hospital, and an appeal dated February 12, 1974, and signed by six leading intellectuals including Sakharov, which reached the West via the *Samizdat* network, speaks of the "appalling conditions of humiliation, persecution and physical suffering" in which he is held. Intensive "treatment" with haloperidol has caused a "sharp deterioration in his health, extreme exhaustion and continuous shivering, weakness, swellings, spasms, and loss of appetite." By that time he could no longer read, write letters or take advantage of the hour's exercise permitted to confinees. All requests by his wife for information on her husband's state of health, and treatment were refused.



Plyushch : *"at the border of death"*

Shortly after this appeal was drafted, an International Committee of Mathematicians for the Defence of Shikhanovich and Plyushch was set up in Paris. (Dr Yurii Skhikhanovich is a Moscow logician who was being held on charges similar to those against Plyushch.) After a number of requests to the Soviet embassy in Paris for news of the two mathematicians, the Committee was informed that the diplomats were "inadequately informed" in these matters, but that the Soviet Union never used confinement in a mental hospital as a punitive measure, and that the "special" hospitals such as that in which Plyushch is confined were established so that scientists could receive especially good treatment and care. Later requests produced the 'information' that Plyushch was dismissed for negligence in his work and for having lost departmental documents. After which he made no attempt to find further work, but engaged in writing and circulating anti-Soviet material. During the judicial enquiry into these activities he was found to be suffering from schizophrenia and was still (March 25, 1974) in need of medical treatment. A reexamination of Shikhanovich "in order to consider the possibility of terminating his course of treatment" was scheduled for March, and in June, he was in fact released, apparently in response to the Committee's pressures.

But Plyushch remains in confinement, perhaps on account of his Ukrainian nationality. Ukrainian dissidents, with their stress on equal rights for minority nationalities (and in particular for their own 50-million-strong 'minority') are always viewed considerably more severely by the Moscow authorities, lest nationalism should lead to a revival of Ukrainian separatism. (The Ukrainian SSR, like the other Union republics, does, of course, possess on paper the right to secede from the Soviet Union.)

Whatever the reason for his continued confinement, the conditions in which he is held according to the appeal of last February, have brought him "to the border of death", and even if he survives there is a real danger of irreversible psychological and intellectual damage.

It is not surprising, therefore, that appeals should have been made to the Vancouver Congress, both from the Paris Committee and directly from Sakharov, to "pass a resolution in Plyushch's defence, and to take all possible measures to save him." What is new is the suggested means of protest and the boycotting of uninvited papers. A great deal may be said in favour of international academic exchange with the Soviet Union, as a means of fostering *detente* and of opening channels through which pressures can be exerted, when necessary, for the defence of academic freedom. However, the long-standing Soviet practice of sending substitutes to international conferences must inevitably tend to vitiate these advantages. The recent appeal on behalf of Leonid Plyushch, if the proposed boycott becomes a regular reaction, may put an end to the high-handed attitude of the Soviet authorities in denying visas to invited delegates. ☐

● News has just been received that Moscow physicist Viktor Polskii, who has been trying for four years to obtain an exit visa to emigrate to Israel, is to be brought to trial on Friday September 20, 1974. Dr Polskii who was dismissed from his post as Director of an X-ray Introscopy laboratory in 1971 after applying for his visa, has several times been threatened with trial and imprisonment, if he would not withdraw his application.

In March, 1974, the authorities received an unexpected stroke of luck when a 19-year-old girl tried to commit suicide by throwing herself under Polskii's car, following a quarrel with her parents. Although she and her parents at first admitted the suicide attempt, they later changed their testimony and now claim that she simply ran across the street. Dr Polskii is therefore faced with prosecution on a charge which carries a maximum sentence of "deprivation of freedom for three years".

The trial, at first scheduled for August 15, was postponed indefinitely, on the grounds that Polskii needed an operation for hernia. There was some speculation that this was a diplomatic retreat on the part of authorities, especially since it seemed that Dr Tsibin, who admitted the girl to hospital following the incident, was prepared to substantiate that she did, in fact attempt suicide. It was felt that the postponement would lead to the charge being quietly dropped.

This, however, is not the case. The trial is to go forward, and the timing of the announcement, during the Jewish High Holidays, seems to carry considerable overtones of propaganda to discourage others.

Professor F. Janouch, of the Niels Bohr Institute, Copenhagen, writes on the plight of the Czechoslovakian academic

PROFESSOR Peleska's letter to *Nature* (July 12), a shocking document, is but one example of the great devastation of the Czechoslovak science and culture, which started back in 1969-70. It is almost impossible to collect all cases of this continuing persecution and one could hardly find a journal or editor willing and able to publish the endless lists of tragedies and frustrations of Czechoslovak scholars and intellectuals.

Professor Burhop's comments (August 9) are too mild and indecisive. No scientist is against international cooperation in science. But what meaning has such cooperation *per se* without any conditions? I am afraid that the damage caused by closed mouths and eyes (which, in fact, is the price for such unconditional "cooperation") is much greater than possible effects.

Professor Burhop reported two further cases of persecution of scientists. The first one—of Ferreiro de Alencar—belongs to cases of judicial persecution, the second—the dismissal of Holzer because of his membership to the Communist Party—belongs to what may be called extrajudicial persecution.

To the first category of judicial persecution of scientists several examples from my country Czechoslovakia could be added. Let me give here only the names of historians Professor Hubl and Dr Tesar, and a psychologist, Professor Sabata, all these are serving sentences exceeding six years for their political activity. It will no doubt be very useful and it will certainly only increase the prestige of WFSW if they would show more initiative in collecting, publishing and condemning such cases of judicial persecution no matter where and when they may happen.

The extrajudicial persecution of scholars and intellectuals is widespread throughout the world, in my country at present it has the dimension of a national catastrophe. The numbers of Czechoslovak intellectuals fired for taking stands, for expressing and defending their opinions, who cannot work in their field (or have difficulties with their self realisation for these reasons) are perhaps being measured now by tens of thousands—too many for a country of 14 millions which can assert itself solely by the extent to which it can contribute to the common culture and is able to help others with experience and knowledge.

I join the protests against the dismissal of Holzer. It seems to me, however, and this is not properly understood in Western countries, that his dismissal, however tragic, does not prevent him from continuing his scientific career, does not prevent him from publishing his papers or books, does not concern the very existence of himself and even of members of his family.

The extrajudical persecution in so-called socialist countries, with the absolute monopoly of political power concentrated in one party or even in a very small group of people, with strict censorship of all mass media, with nationalisation of all branches of education, science, culture, trade, industry, administration and mass media, is incomparably more severe and effective. A man, subjected to extrajudicial persecution in these countries, has no other choice than to take unqualified, manual work. With few exceptions, which may be counted on the fingers of one hand, Czechoslovak intellectuals have no possibility of leaving the country to find work abroad. Their children are deprived the right of education at schools and universities. Their papers and books cannot be published. Let me mention here the shameful instruction issued by Czech Education Minister J Havlín on May 19, 1972, according to which "it is not permitted to publish authors, who have been expelled from the Communist Party of Czechoslovakia, have been dismissed for political reasons from the university or emigrated illegally abroad." I have tried to protest against this, in my opinion, unconstitutional and immoral instruction. The Prosecutor General of the Czech Socialist Republic did not, however, find it necessary to intervene over Havlín's instruction. Such practice, hence, has to be considered as legal in my country.

The consequence of extrajudicial persecution is the isolation from science, which is in our days of rapid development and frequent changes in all fields of science equivalent to scientific disqualification and consequent intellectual death.

Let me point out yet another aspect of Professor Peleska's case: preventing a physician from performing his profession, developing new methods for the treatment of people, easing their suffering, and saving human lives is a crime against humanity—the worse crime because it is covering itself with an ideology which pretends to be the most progressive and most humane one.

The WFSW, no doubt, is aware of the fate of one of the most renowned Czechoslovak scientists, microbiologist Academician Ivan Málek (one of the WFSW founders and for a long time its Vice-President). In autumn 1973, Professor Málek lost his position at his institute and is now being deprived of any possibility of continuing his scientific work. Even his library and his scientific archives have been thrown out of the institute, which Málek founded, built up and directed for almost 20 years. Why does the WSFW remain silent in the fact of such intellectual barbarism? We are living in an open world, and only open, public stands, protests and statements are meaningful and helpful. Intellectual freedom is indivisible, and if world scientific and intellectual organisations continue their ostrich politics with respect to the fates of their persecuted colleagues, they will not only lose the moral right to speak on behalf of the world intellectual community, they will lose their intellectual freedom too.

correspondence

Whales

SIR,—With reference to the article "The Unendangered Whale" (August 9), we feel that it is most important to point out that the opinions expressed by Dr Ray Gambell are by no means universally accepted

Gambell states that the "concept of species management is now operative" But 'concepts' do not ensure that the whales will be harvested rationally There are many more practical factors which come into play besides scientific concepts of species management At the International Whaling Commission (IWC) meeting last June, some of the recommendations of the Scientific Committee (of which Gambell is a member) were overruled in the plenary session in favour of higher quotas It is also worth noting that there is still time for Japan and the Soviet Union to opt out of any of the management policies under the 90-day rule In fact, there is no real need for Japan to opt out of any of the decisions made by the IWC as she owns dummy companies (joint ventures, flags of convenience) in countries which do not even belong to the IWC

Gambell also points out that it also "remains to be seen just how well the scientists can resist the political and economic pressures" Past experience (of which there is much) suggests that they are unlikely to be able to resist such pressures Some Japanese whaling scientists have admitted that they are torn between the demands of the Japanese whaling industry and the necessity for sound conservation measures

The reason why many experts are now opting for a 10-year moratorium on commercial whaling is because they have begun to realise how little they know about whales and the effect of whaling on their populations Gambell is not one of these scientists, but he has conceded that "there is much research needed" It has also recently been brought to Gambell's attention that perhaps the biomass of whales should be studied, not just their numbers Had the scientists looked at the wider effects of whaling–other than those demanded by the industry-dominated IWC–they would have seen that the weight of sperm whales, for example, has declined rapidly during the past 30 years, and that in terms of biomass is probably well below maximum sustainable yield (MSY) There is also reason to believe that the total biomass of baleen whales in the Antarctic is also well below MSY We feel, therefore, that Gambell is rather premature in reporting that the whales, situation is satisfactory

Fortunately the British government is still in support of the complete moratorium on commercial whaling despite Gambell's advice

Yours faithfully,

JOHN A BURTON
ANGELA KING

Friends of the Earth Limited,
London W1

Biohazards and the law

SIR,— Brian Ford (*Nature*, August 2) may be right in saying that there is not enough *statute* law about biohazards, and his suggestions for future legislation are certainly valuable But statutory codes of practice cannot by themselves solve the problem Sooner or later, by accident or design, someone somewhere will ignore them What is important is what happens then

The offender may be fined or sent to prison, but that will provide little comfort for the victim Far more important is the civil remedy which the *common law* (i e the law made by the judges) has designed for such cases, centuries before Parliament started to take an interest in this field

Under the rule known as Rylands v Fletcher, anyone who for his own purposes keeps on his land anything likely to do mischief if it escapes is answerable for all the damage which is the natural consequence of its escape The liability does not depend on negligence all the victim has to prove is that the mischievous thing was kept there, escaped, and caused him damage, and it avails the defendant nothing to show that he took all possible care, or even that he did not know the thing was dangerous

There are cases in the books, as recently as 1928, which apply this rule to poisonous substances I know of no reason why the courts should not apply it to living organisms—though, if they were held to be animals, they would now come under the Animals Act 1971, which replaces common law rules broadly similar to the rule in Rylands v Fletcher

If every owner, director and operator of a laboratory which kept human pathogens on its premises realised that in the event of an escape he would be absolutely liable to compensate all victims for all the damage they suffered, the standards of care to prevent escape from those laboratories might well be higher, and more strictly observed, than any new legislation could expect to achieve

Yours faithfully,

PAUL SIEGHART

Council for Science and Society,
6 Gray's Inn Square,
London

Megalithic alignments

SIR,—As one who has recently reviewed several of John Michell's books at some length *Men, myths and megaliths*, Thames and Hudson, in the press), I read his letter (August 23) with considerable interest

To anyone who has made the effort to wade through the corpus of his bizarre geomancies, it comes as no surprise that most archaeologists have declined the privilege of reviewing his latest efforts I suspect that many regular readers of *Nature* will be unfamiliar with Mr Michell's books and his ideas Basically he is a neo-Straight-tracker, a disciple of Alfred Watkins (*The Old Straight Track*, Methuen, 1925) who believed, after a mountain-top 'vision' one summer afternoon in about 1920, that Britain was networked with alignments (including Megalithic ones) which had been laid out in premeditated fashion by some ancient race

But Michell is also a self-confessed flying-saucer enthusiast, an admirer of Piazzi Smyth's Pyramid theories, Mrs Maltwood's Glastonbury zodiac and several other claptrap cults which provide the right kind of fuel for his own romances

Michell's technique, rather cunningly, is to throw in any semi-respectable or respectable material in attempts to add verisimilitude to his own For example, Fred Hoyle must be 'delighted' to find that Michell is a staunch supporter of his 'Stonehenge drum-beat theory' (about 1966) In *The View Over Atlantis* (Abacus, 1973) he writes (page 183) 'Professor Hoyle in an article in *Antiquity* suggested that the men who built Stonehenge may have communicated over long distances by beating drums ' and then Michell splices in his own ideas ' it was with drums, songs and the clash of cymbals that magical flight was achieved according to legend

Stone levitated by sound could become a flying chariot moving along the line of a certain magnetic intensity, whose course was marked out on the ground by alignments of stones and earthworks, linked by raised causeways and rides through the forest With the Earth's magnetic [sic] field regulated and the streams that run through it diverted to conform to straight lines, the stone craft and its navigator could float from centre to centre, picking their way through a canal network of alternating currents and choosing the level of intensity to which their vibrations were attuned'

Geophysicists also please note!

Yours faithfully,
P Lancaster Brown

Beaconsfield, Bucks

Closely observed trains

Sir,—The facile comment under the photograph on page six of your journal of the 6th of September, prompts me to raise a few points with regard to the present state of magnetic suspension technology in this country and abroad

How good at speed? Your correspondent gives the answer herself on page seven The 45 mile track being constructed in Germany is for a high speed system using controlled electromagnets The proposed speeds are 500 km/h Krauss Maffei have no problems that they cannot overcome on eddy currents up to 400-450 m p h Our own tests confirm the Krauss Maffei measurements made in 1972 when they ran their vehicle at over 150 k p h Nobody in their right minds and connected with the d c electromagnet suspension technology would see any problems at speeds as low as 50 m p h The problems at Toronto are those of linear induction motors and the inverter drive for them It is also worth noting that work on the French Aerotrain has come to a halt and discussions have taken place between the French and German authorities on a joint programme of development of the controlled electromagnet systems, for intercity and transeuropean network So much for the misplaced Sussex claims

There is in my opinion, an obsession with high speed and ground transport which in turn leads to a complete blinding of the technical problems (never mind the social problems) of high speed vehicles Guidance of such vehicles is not a matter of showing that 20lb aluminium plates or one ton vehicles in laboratories have stability The turning moments on a 30 ton, 100 passenger vehicle are of the order of 40000 N M The aerodynamic drag alone is more than 2000 kW and therefore that is the size of the required linear motor (The suspension power using d c electromagnets is 30 kW)

No-one claims to have solved the problem of collecting this sort of power at 300 m p h from what your correspondent calls "normal a c mains" It is a little bit more difficult than plugging a shaver into a socket If two of these trains cross each other this will impose an impulse load of more than four MegaWatts on the area generating board Is the generating board going to be happy about this happening at three min intervals? Are the high speed vehicles to run from A to B only or are they intended to change routes at speed? If yes, has some consideration been given to each proposal including the tracked hovercraft? The cost apart, these are some of the more formidable problems of high speed transportation vehicles There are a few mundane ones like response times of the guidance systems, an accurate estimate of propulsion and suspension powers (no-one gets this as a bonus, as has been claimed), dependence of guidance and suspension forces on speed, power factors and efficiencies of the proposed equipment to name a few

My team and I have lived with these problems for the last five years and are in a position to offer experimental evidence on suspension, guidance and propulsion aspects (including eddy current drag) We do not claim to have solved the world's transportation problems or to offer the ultimate system The Germans have been developing their d c electromagnet systems for the last three years and are going ahead with the same technology for high speed application The Japanese on the other hand have run into serious problems of guidance on their cryogenic system We could investigate our system on the Brighton seafront to find out (a) whether it is possible to implement it in our cities and (b) whether a high speed system is feasible Alternatively, we could always buy the finished article from Germany say, in 1985 or 1990

The choice of Brighton seafront is not so gimicky as it appears Environmentally it is one of the toughest locations one could choose with corrosion and high winds presenting a severe test of any installation

Whether Professor Laithwaite's magnetic river system is superior to all others can only be judged when he produces enough technical data and hardware to justify his claims Even if it is, his problems will only then begin A high speed transport system cannot be developed on a domestic market A transeuropean system is likely to be German-French dictated Perhaps Sussex claims are not all that misplaced!

Yours faithfully,
B V Jayawant

University of Sussex,
Applied Science Laboratory,
Brighton

Time for a change?

Sir,—In *Nature*, Vol 250, August 30, 1974 was published a paper by Charles E Chaffey on a time-measuring system based on SI units Obscurantism for its own sake is admirably displayed in this paper The time-system postulated is unintelligible, having no frame of reference, and being unrelated to reality—days, lunar months and years, which exist on this planet in the absence of all arbitrary measurement

The author states that his aim is "the elimination of timers graduated in diverse units", presumably without regard to convenience or intelligibility The SI second, though defined differently, is still based upon day length divided by 24×60×60, therefore is irrational in terms of the decimal system (the division of the clock dial into 12 or 24 *is* rationally related to the geometry of the circle) Surely, if a so-called 'rational' system is to be adopted by scientists, then it should be the metric clock and the French Revolutionary Calendar (now being the last month of 182)

Incidentally, what is the starting point of C Chaffey's calendar? The Christian era? Surely not Why not Sir Isaac Newton's birthday? This would have the merit as being the same day as Christmas, and would remind all scientists of this great alchemist/astrologer/theologian who founded our new religion

Yours faithfully,
Nigel Pennick

Trumpington,
Cambs

Group papers

Sir,—Alan Mackay's proposal (*Nature* 250, 698, 1974) that papers should be published in the name of the research groups rather than of the individuals, deserves serious consideration in spite of his outdated references (to St Matthew and Church of England Articles) This procedure has in fact been encouraged in China The prime purpose of scientific communication is definitely better served by an improved standard, and decreased number, of publications (have you not read any recent issue of J Irreg Results?) No author or authors of any paper can claim exclusive credit for it why, otherwise should there always be acknowledgements? Under the new arrangement, individuals will still get the 'carrot' by belonging to a group which publishes a lot of good papers, and they won't miss the 'stick'

Yours faithfully,
T B Tang

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news and views

Spectroscopy of organic molecules in solids

THE spectroscopist is interested in studying energy levels, the probabilities of transitions between levels, and the lifetimes of excited states Because of the low volatility of most organic compounds their spectra are normally observed by using solutions in a liquid or vitreous solvent The ultraviolet and visible absorption and emission spectra of solutions are, however, generally very broad and the structure that may arise from molecular vibrations is absent This loss of information can seriously reduce the value of observations of the electronic spectra of organic molecules

In a dilute vitreous solution, the interaction of a solute molecule with its local solvent environment perturbs the energy levels of the solute This interaction is different at different sites in the glass so the sample comprises a range of distinguishable solute molecules, the spectrum of such a sample is said to be 'inhomogeneously broadened' In a liquid solution, all solute molecules experience the same environmental influences (when an average is taken over the time that the molecule exists in the particular energy level) and the spectrum is 'homogeneously broadened'

The absorption spectrum of a glassy solution is broad, even at very low temperatures when the vibrational motion of the solvent is suppressed Its emission spectrum is also broad when the excitation is due to a broadband arc source The emission, or fluorescence, spectrum is caused by transitions from the lowest vibrational level of the electronically excited molecule to various vibrational levels of the ground electronic state (higher vibrational states of the electronically excited molecule generally decay very rapidly by non-radiative mechanisms to the lowest level, so the vibrations of the excited molecule are not normally seen in emission) Vibrational structure is not seen in such an emission spectrum if the variations in the energy-level differences due to site changes are larger than the vibrational spacings, and this is the usual situation for organic species

On page 215 of this issue of *Nature*, Eberly, McColgin, Kawaoka and Marchetti of the University of Rochester and the Eastman Kodak Research Laboratories in Rochester, New York, describe a method for obviating the effects of inhomogeneous broadening of the electronic spectra of organic solutes in vitreous solvents They employ a narrow-band laser source and observe vibrational structure in emission spectra Their success depends on the fact that only a small fraction of the solute sites in the glass is in resonance with the narrow-band source, leading to a selection of a particular class of solute molecules (those whose environments are sufficiently similar to let them absorb the incident radiation) Their technique is particularly effective when the incident radiation has the frequency corresponding to the energy difference of the lowest vibrational levels of the excited and ground states, the 0–0 band At higher frequencies, variations in the site may bring different vibrational levels of the electronically excited molecule into resonance Actually a number of ' extra" emission lines were seen from a 10^{-4}M solution of phenanthrene in a glass at 4 2 K when the incident frequency was 0 24 eV (1,900 cm^{-1}) above the centre of the 0–0 absorption band

The successful elimination of inhomogeneous broadening of the spectra of dilute solutions of organic molecules in glasses is reminiscent of the Lamb-dip, of a sharp form of two-photon absorption in the high-resolution spectroscopy of gases, and of the spin-echo experiment in nuclear magnetic resonance The Lamb-dip and sharp two-photon absorption may be seen in gases in intense radiation in a standing wave, when only those molecules with negligible velocity in the direction of propagation interact with both the forward and reflected beams, Doppler broadening is thus eliminated Hahn's spin-echoes arise through a reversal of the precession of magnetic moments through the application of a second pulse, the magnetic field inhomogeneities speed up or slow down both the forward and reverse precessions, leading to a coincidence, or 'echo', in the detector and the elimination of the broadening due to field inhomogeneities

The Rochester experements have yielded structure in the emission spectrum of naphthacene in a glass at 4 2 K This structure has been analysed to give vibrational frequencies of the molecule in its ground electronic state Though this is interesting and promising, and probably capable of wide application because of the availability of narrow-band tunable dye lasers, it should not be forgotten that these frequencies are easily obtainable from infrared and Raman spectroscopy DAVID BUCKINGHAM

Nude mice

SINCE their original description in 1966 (Flanagan, *Genet Res*, **8**, 295), nude mice have spread through the immunological world at a remarkable pace While mice heterozygous for the mutant nude gene (*nu*/+) are apparently normal, homozygotes (*nu*/*nu*) are hairless (hence their name) and have hypoplastic thymus glands (Pantelouris, *Nature*, **217**, 370, 1968) The thymus abnormality leads to a marked deficiency in thymus-derived (T) lymphocytes and nude mice are rapidly replacing thymectomised mice as models of T cell deprivation and as a source of relatively pure B lymphocytes for *in vitro* studies In many laboratories the *nu* gene is being back-crossed onto a variety of inbred backgrounds thereby increasing their usefulness for cell and tissue transfer experiments The T cell deficiency in nudes make them highly susceptible to infection (but apparently not to cancer) and they survive poorly in conventional animal houses Thus they are usually produced by mating heterozygotes

The extent of the T cell deficiency in nudes has remained controversial They are usually referred to as being 'congenitally athymic' but this is clearly a misnomer in view of the presence of a thymus gland in all nudes so far studied They have been shown to have T cell precursors which can repopulate a normal thymus (Wortis *et al*, *J exp Med*, **134**, 681, 1971) and develop into immunocompetent T lymphocytes (Kindred and Loor, *J exp Med*, **134**, 681, 1971) and several reports have suggested the possibility that small numbers of T cells are present in at least some nudes For example, approximately 1% of lymphoid cells in nude spleen and lymph nodes label directly with fluoresceinated alloantibodies directed against the θ (thy-1) alloantigen, a T cell marker in mice (Raff, *Nature*, **246**, 350, 1973) Although θ is expressed on some neural cells, epidermal cells, and fibroblasts, in addition to thymus and T lymphocytes, there is no evidence that it is found in readily detectable amounts on B lymphocytes, granulocytes, macrophages or other cells in lymphoid or haemopoietic tissues (Owen *et al*, *Nature*, **249**, 361, 1974) Thus it seems likely that these few θ^+ lymphoid cells in nudes are T lymphocytes, although their origin (host or maternal, thymic or extrathymic) and functional capabilities remain unclear

On page 229 of this issue of *Nature*, Loor and Roelants report the presence of another population of θ^+ cells in nude spleen which are distinguished from normal T lymphocytes (and those described previously in nudes) by having such small amounts of θ on their surface that they can be detected only by indirect immunofluorescence, particularly with an absorbed rabbit anti-mouse brain serum which appears to have anti-θ-like specificity These cells lack detectable surface immunoglobulin (a marker for B lmphocytes) and make up approximately 20% of nude spleen cells, but are present in very small numbers in nude lymph nodes and in lymphoid tissues of normal mice The identity of these cells is unknown and it seems premature to refer to them as T cells, which implies their migration from the thymus Loor and Roelants make the intriguing suggestion that these cells may be precursors of T cells, which perhaps have followed some aberrant differentiation pathway due to the lack of a normal thymus This is an attractive possibility in view of the recent evidence that one can induce the appearance of θ and other thymus alloantigens in cells lacking readily detectable amounts of these antigens by exposing them to a variety of substances (such as dibutyryl cyclic AMP and thymus extracts) for 1–2 h *in vitro* (Scheid *et al*, *J exp Med*, **138**, 1027, 1973)

Many pieces still are missing in the nude puzzle, the most important being the role of the presumptive wild-type protein in normal thymus and hair development The extent of the T cell deficit and the origin and nature of the θ^+ cells in nude lymphoid tissues may be answered, in part at least, by studying nude mice born in *nu*/*nu* parents, rather than of *nu*/+ parents as has been the case thus far Only in this way can the role of maternal and normal litter-mate T cells and thymus humoral factors on nude embryos be determined If such mice lack θ^+ cells in their lymphoid tissues it would establish once and for all the essential role of the normal thymus in their production

MARTIN C RAFF

When the ice age seemed to end

IN the opening years of this century a sequence of muds and clays exposed on the Island of Sjaeland, Denmark was described In two clay layers was an abundance of remains of the arctic alpine plant, *Dryas octopetala*, considered to be indicative of cold conditions The intervening mud contained remains of a more temperate flora including birch trees The sequence was thought to represent a climatic oscillation at the close of the last (Weichselian) glaciation, and the temperate interstadial was termed 'Allerød' after its site of origin Subsequently this sequence was found over a wide area of north-west Europe and the advent of radiocarbon dating has made it possible to assign tentative dates to the lithological boundaries West (in *Pleistocene Geology and Biology*, Longman, London, 1968) quotes the following values, which are averages of many dates

Older Dryas/Allerød Interstadial	11,950 b p
Allerød Interstadial/Younger Dryas	10,750 b p
Younger Dryas/Preboreal	10,250 b p

Further investigations in Denmark, however, led to complications in this basic scheme At Bøllingso a sequence was found in which the clays of the Older Dryas period were interrupted by a mud layer, indicating an interstadial predating the Allerød Similar sequences have now been found in Germany, Holland and Norway, and several radiocarbon dates are available Van der Hammen *et al* (*Geologie Mijnb*, **46**, 79, 1967) date the episode at 12,400-12,000 b p in the low countries, but a Norwegian date (Chanda, *Årbok Univ Bergen*, No 1, 1, 1965) places its openings at 12,650 b p In some British pollen diagrams there is evidence of a minor vegetational change in pre-Allerød times, for example at Haweswater (Oldfield, *New Phytol*, **59**, 192, 1960) and, more convincingly, at Tadcaster Yorks (Bartley, *ibid*, **61**, 277, 1962) but neither site has been radiocarbon dated

A kettlehole site, now beneath Blelham Bog near Windermere, was found to have two organic layers in its late Devensian (≡late Weichselian) sediments Pennington's pollen diagram (in *Studies in the Vegetational History of the British Isles*, edit by Walker and West Cambridge, 1970) of these layers shows a proportional increase in tree birch pollen during the lower organic layer, but the radiocarbon date obtained (14,330±230 b p) is considerably older than the classic Bølling interstadial Subsequently Pennington and Bonny (*Nature*, **226**, 871, 1970) have confirmed the dating of this layer with a further date of 14,280±230 b p and have also clarified the vegetational events of this period by means of absolute pollen sedimentation determinations (see Davis and Deevey, *Science*, **145**, 1293, 1964) They found that the pollen input rate of the lower organic layer was similar to that in the adjacent clays, the apparent rise in birch pollen was a statistical artefact resulting from the use of proportional expressions Thus in Britain there is still no well authenticated and dated interstadial equivalent to the continental Bølling which is separable and distinct from the subsequent Allerød interstadial Bartley's Tadcaster site remains the most hopeful contender

At Colney Heath in Hertfordshire there is a peat which may have been transported by a glacier and buried in detritus Godwin (*Proc R Soc*, **B150**, 258, 1964) described an arctic flora from this peat and dated it at 13,560±210 b p This material thus postdates the Blelham lower organic layer, but gives no indication of an improvement in climate

Datings of the opening of the supposed Allerød interstadial have usually depended upon lithological changes, in particular the replacement of minerogenic sediments by organic ones Early dates of this boundary showed considerable consistency and were also in close agreement with continental dates Godwin and Willis (*Proc R Soc*, **B150**, 199, 1959) quote such dates as 11,942±120 b p (Lunds, Yorks), 11,880±225 b p (Keith, Banffs) and 11,870±120 b p (Windermere) Subsequent dates have not shown this degree of consistency, for example at Loch Droma, Ross and Cromarty, a date of 12,810±155 b p was obtained (Kirk and Godwin, *Trans R Soc Edinb*, **65**, 225, 1965) This date was very difficult to explain since it predated even the Bølling and yet gave all indications, lithological and palynological, of being the commencement of the Allerød Other similar dates have now been obtained from a variety of sites, particularly in Scotland Bishop (*Trans J Proc Dumfries Galloway nat Hist Antiq Soc*, **40**, 117, 1963) gave a date of 12,940±250 b p to the opening of the interstadial at Lockerbie, Dumfries, Sissons and Walker (*Nature*, **249**, 822, 1974) give dates of 12,750±120 b p for kettle hole deposits near Callander, Perth, and 11,290±165 and 13,151±390 b p for Loch Etteridge in the Grampians of Scotland At the latter site, the younger date corresponds to the commencement of gyttja sedimentation and the older date refers to the underlying clay gyttja Pennington and Bonny date the climatic improvement (as evidenced by a considerable increase in absolute pollen influx) in Blelham Bog at between 12,700 and 12,950 b p Simpkins (*New Phytol*, **73**, 605, 1974) has recently published a date of 12,050±250 b p for the base of organic sediments from a kettlehole at Glanllynnau, North Wales, but Coope and Brophy (*Boreas,* **1,** 97, 1972) have obtained a date of 12,556±230 b p for the same layer using only the macrofossil fruits of cyperaceous plants for the dating and thus avoiding contamination from penetrating roots At Glanllynnau, the basal organic sediments contain little tree birch pollen, the expansion of this constituent occurring 10 cm above the clay/gyttja transition Juniper pollen is poorly represented throughout, but there is a trace of expansion within these basal layers An analysis of the fossil Coleoptera of this site by Coope and Brophy gave evidence of climatic improvement well below the lithostratigraphic change, at 12,850±250 b p This improvement was reflected neither in the lithology nor in the relative proportions of the pollen assemblage at that level

This collection of dates and observations is leading some researchers, particularly Pennington, to propose that some of the lithological and biological changes which have been taken to represent the commencement of the Allerød interstadial, *sensu stricto,* are in fact indicative of the climatic amelioration which gave rise to the Bølling sediments on the continental mainland Climatic improvement may have begun by 13,000 b p but lithological changes in some sites was delayed for a thousand years In Britain the two interstadials seem to have run into one, particularly in the northern and western parts of the islands Presumably the oceanicity of the British Isles has influenced the degree to which climatic thesholds have been successively crossed, as a result, the response of the biota and any fluctuations in the nature of sedimentation have been dampened in the amplitude of their oscillations It would seem that the search for a Bølling in the west of Britain is at an end

Faunal evidence for a decline in temperature during the interstadial has been found as early as 12,000 b p by Coope *et al* (*Palaeogeogr Palaeclimatol Palaeoecol*, **10,** 87, 1971) Lithological evidence for the close of the interstadial and the onset of solifluction is dated 10,828±185 b p at Scaleby (Godwin, Walker and Willis, *Proc R Soc*, **B147,** 352, 1957) and at Loch Etteridge, Sissons and Walker obtained a date of 10,765±120 b p The final close of the Allerød thus seems to be a well marked occurrence, the date of which may vary slightly from one locality to another dependent on local conditions such as relief

The end of the cold Younger Dryas period was dated by Godwin *et al* to 10,257±350 b p at Scaleby Moss, but there is a problem of lithological and biological definitions of this boundary At Scaleby the date refers to the first rise in the proportion of juniper pollen Some other dates for this change are 10,490±160 (Blelham, Pennington and Bonny), 9,590±170 (Bigholm Burn, Dumfries, Moar, *New Phytol*, **68,** 433, 1969) 9,508±200 (Red Moss, Lancs, Hibbert *et al*, *Proc R Soc* **B177,** 161, 1970) and 9,660±105 (Slieve Gallion, Co Tyrone, Pilcher, *New Phytol*, **72,** 681, 1973) As Smith and Pilcher (*New Phytol*, **72,** 903, 1973) have pointed out, this biologically defined horizon is evidently diachronous within the British Isles The use of lithological features (that is, the replacement of clay by primarily organic sediments) is equally variable, however Birks (in *Past and Present Vegetation of the Isle of Skye,* Cambridge, 1973) gives dates of 9,420±150, 9,482±150 and 9,655±150 b p for the base of Flandrian muds in Skye Sissions and Walker also have a late date, 9,405±260 b p for a similar horizon at Loch Etteridge Evidently the conditions necessary for lithological change were satisfied at different times in different parts of the country, depending upon local climate, relief, geology and so on The definition and dating of the close of the late-Devensian period and the opening of the Flandrian thus remain rather obscure

With the increasing number of radiocarbon dates and biological and lithological analyses of late-Devensian deposits in Britain, the complex underlying climatic changes within this unstable period are beginning to be understood It is becoming clear that simple threefold division of the period is a gross and rather misleading misrepresentation of the diverse temporal and spatial conditions found in the British late-Devensian PETER D MOORE

New Miocene polarity scale

from Peter J Smith

THE statistical analysis of geomagnetic field reversals, begun by Cox (*J geophys Res*, **73,** 3247, 1968) and later developed further by Blakely and Cox (see, for example, *J geophys Res*, **77,** 4339, 1972), seems a complicated business to non-mathematicians Fortunately however, most of the conclusions emerging from it are relatively easy to understand and offer an interesting example of the predictive power of statistics where a data set is incomplete and likely to remain so for some time The problem with the data set in this case is that, although relatively long periods of single polarity may be detected and measured with reasonable accuracy, the lengths of very short polarity intervals lie within the errors of the dating technique This, coupled with the fact that short intervals are intrinsically unlikely to be represented by very many magnetised rock samples, makes it difficult to investigate experimentally any variations in field behaviour (for example, reversal frequency) with time

Previous statistical work on the geomagnetic time scale has indicated that field reversals apparently ocurred randomly throughout the Cainozoic and may be described by a Poisson probability model with an average reversal frequency λ But λ seems to vary with time, most markedly during the Miocene The frequency of known reversals over the past 45 million years (Myr), for example, was 3 0 Myr^{-1}, whereas the period 22 7–7 3 million years ago had an anomalously low average reversal frequency of 2 5 Myr^{-1} So the question is did the reversal rate really decrease over this limited period, or do other reversals (probably linked with short polarity events) remain to be discovered?

To find out, Blakely (*J geophys Res*, **79,** 2979, 1974) has analysed 18 total field profiles from the north-east Pacific, the east Pacific rise and the Indian–Antarctic ridge using the signal-averaging technique previously developed by Blakely and Cox, a technique by which profiles are combined and normalised to a common average spreading rate Among the important results to emerge or follow from this analysis are the following

A comparison of the combined (stacked) profile from 14 north-east Pacific profiles with a computer-generated model profile from the time scale of Heirtzler *et al* (*J geophys Res*, **73,** 2119, 1968) shows that for the period 22 7–7 3 Myr there are eight short-wavelength magnetic anomalies present which are not predicted by the previously published time scale In particular, anomaly 5, originally thought to be a single constant polarity epoch lasting for 1 15 Myr, is now seen to comprise five short-wavelength anomalies, thus confirming the result of Cande and Labrecque (*Nature,* **247,** 26, 1974) Additional data from four east Pacific rise and Indian–Antarctic ridge profiles (areas distant from the north-east Pacific) confirm the presence of the eight extra anomalies on a worldwide basis

A plot of average spreading rate for the 14 principal profiles taken together (constructed from data obtained incidentally in the main analysis) suggests that the rate varied often, and often quite violently, over the period 22 1–7 6 Myr ago Between 19 3 and 18 5 Myr ago, for example, the spreading rate apparently decreased from 47 7 mm yr^{-1} to 21 8 mm yr^{-1}—a surprising deceleration within only 0 8 Myr Apparent spreading rates for the other two areas of study taken separately fluctuate with time in a similar manner, implying further that spreading rates were changing coherently throughout the world The face-value interpretation of these results is that, during the Miocene, seafloor spreading did indeed proceed discontinuously

There is another possible explanation, however The basis of comparison used by Blakely in his analysis was the time scale of Heirtzler *et al*, a scale which assumes that seafloor spreading was constant at the mid-Atlantic ridge during the Cainozoic and which used the single magnetic profile from Vema cruise 20 in the South Atlantic But this interpretation may not be valid If, instead, it is assumed that the Pacific, Indian and Antarctic plates were each moving uniformly during the Miocene, then the Heirtzler scale must be adjusted For various reasons (for example, that accelerations of entire plates relative to the asthenosphere would have had to have been improbably high at 20 km Myr^{-2} or even 40 km Myr^{-2}) Blakely concludes that such uniformity is likely, or at least the most likely situation given current data As Blakely points out, although many examples of variable spreading rates have been described by other workers, most are from localised areas and do not include evidence for variations synchronised on several plates The assumption of zero acceleration for plates is thus the simplest, but still "tenuous at best"

Nevertheless, using this assumption Blakely goes on to construct a new Miocene time scale which adds 18 reversals to the Heirtzler time scale for the period 22 7–7 6 Myr Sixteen of these reversals are derived from the eight short-wavelength anomalies, and the remaining two are obtained by dividing the positive interval at 13 Myr on the Heirtzler scale into two smaller positive events The revised scale now contains 14 short events lasting less than 0 1 Myr It also has an average reversal frequency of 3 7 Myr^{-1}, which is a significant increase on the previous 2 5 Myr^{-1} At the same time, the new figure is higher than the 3 0 Myr^{-1} rate derived from known reversals over the past 45 Myr, which suggests that there may also be some undetected events outside the more limited Miocene period considered by Blakely

Finally, by comparing magnetic profiles for crustal magnetisation models with real profiles, Blakely is able to estimate the width of the transition zones from one polarity to the other In each of the three models concerned the magnetised crust is regarded as a 0 5 km thick horizontal slab with its upper boundary at a depth of 3 5 km, but the models differ in the width of the band about the spreading centre over which new crust becomes magnetised The best fit to the data from the north-east Pacific is obtained for a model in which the transition between polarities takes place over a horizontal distance of 6 km This figure is, however, highly dependent on the thickness of the magnetic layer, for a layer thicker than 0 5 km, 6 km would be an overestimate

Anti-actin

from Dennis Bray

THE rumour that antibodies cannot be produced against actin lasted about five years Born of one or two unsuccessful attempts to raise antisera to this major protein of muscle, the rumour was nurtured by a familiar process of *post hoc* rationalisation Actin is non-antigenic it was said, because it has such a strongly conserved structure that the small differences between species are not able to provoke an immune response Actin is non-antigenic because it is present in almost every cell of the body so that all animals will have innately acquired tolerance Anybody thus dissuaded from an attempt to make this most useful antiserum, however, has cause for regret For there are now three independent reports of its production

The starting point for two of the reports was the observation that patients with certain aggressive forms of hepatitis have autoimmune antibodies in their circulation Although it was known from work with fluorescent antibodies that these were directed against smooth muscle, the precise nature of the antigens was not known Gabbiani and colleagues (*Am J Path*, **72**, 473–488, 1973) have now examined this by absorption studies and have found that this serum cross reacts with the actomyosin of blood platelets and with the purified component known in platelet circles as thrombosthenin A, but elsewhere simply as actin Concluding from their tests that the serum is essentially anti-actin, the authors used it in immunofluorescent tests to show the distribution of this protein Many non-muscular tissues, including liver cells, brush borders and intestinal epithelial cells, were found to be specifically stained

Stimulated by the same medical findings, and in the laboratory in which they were originally made, Trenchev and colleagues (*Clin exp Immun*, **16**, 125–136, 1974), sought to compare the autoimmune sera with those against various protein components of human smooth muscle These were prepared by conventional methods against muscle myosin, tropomyosin and actin, and used in immunofluorescent tests Once again a constellation of tissues were stained and a correlation between staining and the presence of microfilament arrays was noted, as in the networks of stained filaments seen in HeLa cells The different types of artificially induced antibodies gave slightly different staining patterns but none seemed to resemble the hepatic sera more closely than the others

Quite a different method and a highly original one was used by Lazarides and Weber (*Proc natn Acad Sci USA*, **71**, 2268–2272, 1974) Rather than prepare antisera to conventionally prepared actin, with its persistent complement of other proteins such as tropomyosin, these authors used the bold and very direct approach of injecting material recovered from SDS (sodium dodecyl sulphate) gels The source of their material, mouse fibroblasts, is rich in actin and even a crude fractionation is adequate to give a band on acrylamide gels which is fairly pure The denatured protein extracted from the gels is apparently a potent antigen and able to induce antibodies which cross react with both fibroblast and muscle actin When used to stain the fibroblasts by the indirect fluorescent method this serum gave striking results Brightly stained filaments in a wide variety of patterns are seen, sometimes aligned in parallel with the long axis of the cell or close to the outer surface, at other times arranged radially around central focal points

Taken together these three reports provide good evidence that antibodies to actin can be produced, although the legally minded will not be without reservations In not every case was the purity of the actin adequately monitored—a boring point but important in the light of previous experience—nor were all of the immunological controls carried out Surprisingly, not one of the antisera was tested against adult skeletal myofibrils which surely would give a direct test of their specificity And the use of antisera to denatured proteins is an uncertain business which, again to invoke rumours, may not have such a high degree of specificity as one is used to with native proteins

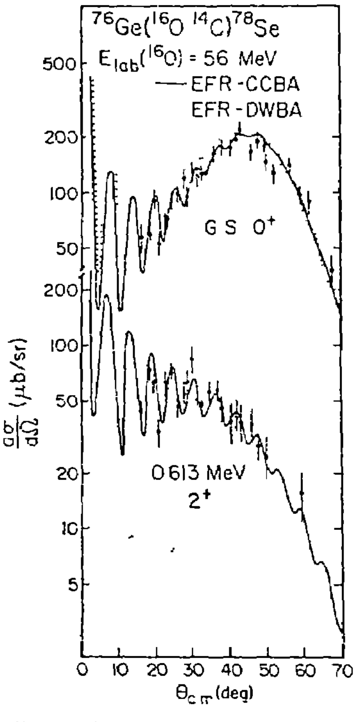
But these are quibbles which will no doubt be resolved in time In general the results are most encouraging, both from a medical point of view as well as that of cell biology The technique of Lazarides and Weber, using the combination of immunofluorescence and SDS gels would seem to be of particular potency One of its strengths is its applicability to structural proteins normally classed as insoluble and almost the first question about such proteins is where are they in the cell? Electron microscopy can give some indication, but is limited in its ability to identify different chemical types and in the area of the cell which it can cover Methods involving antisera and the light microscope are potentially free of both of these restrictions

Two-nucleon transfer between heavy ions

from P E Hodgson

THE theoretical analysis of reactions between heavy ions in which two nucleons are transferred from the projectile to the target has now been developed to a high degree of sophistication The basic distorted wave theory was formulated many years ago and successfully applied to analyse (d,p) reactions But in the case of heavy ion reactions many of the approximations that are made for (d,p) reactions are no longer valid, in particular the use of a zero-range interaction and the neglect of recoil Inclusion of these effects is very complicated and greatly increases the computation time Furthermore it has become increasingly clear that in many transfer reactions involving collective nuclei the contributions from two-step processes that take place through the pre-excitation of the target nucleus or by post-excitation of the residual nucleus must also be included Lastly in heavy ion reactions there are substantial contributions from a large range of incident orbital angular momenta

All these factors have combined to make the whole calculation prohibitively long, even on the fastest computer, if it is carried out in the usual way Recently, however, Low and Tamura (*Phys Lett*, **48B**, 285, 1974) have developed new techniques for evaluating the multidimensional integrals that occur in these calculations, and these have so strikingly reduced the computing time that these calculations are now practicable



Differential cross section for the two-proton transfer reaction $^{76}Ge(^{16}O,^{14}C)^{78}Se$ to the ground and 2^+ excited state of ^{78}Se compared with exact finite range calculations The dotted line shows the one-step distorted wave calculation (EFR-DWBA) and the full line shows the coupled channels calculation (EFR-CCBA) that takes into account the contributions of two-step processes in the exit channel

These new computing techniques have been applied by Tamura, Low and Udagawa (*Phys Lett*, **51B**, 116, 1974) to calculate the cross section for the two nucleon transfer reaction $^{76}Ge(^{16}O,^{14}C)^{78}Se$ to the ground and first excited (2^+) state of the final nucleus Comparison with the measurements of Lemaire, Mermaz, Sztark and Cunsolo shows that the distorted wave calculations are inadequate, even when finite range effects are taken into account (see dotted line in figure) The cross section for the reaction to the ground state shows too much oscillation and is too high at small angles, while that of the reaction to the 2^+ state at 0 613 MeV is far too small

They then took into account the inelastic excitation in the exit channel The residual nucleus ^{78}Se is easily excited into low-lying collective states, so it is possible for the two transferred nucleons to be first added to the target to give ^{78}Se in its ground state, and then for the outgoing ^{14}C nucleus to interact again and excite the ^{78}Se nucleus to the 2^+ state This two-step contribution to the reaction adds to the direct contribution when the two nucleons are transferred so as to produce the ^{78}Se nucleus immediately in its 2^+ state There is also a small effect on the cross section of the reaction to the ground state, for this similarly has a two-step component that goes first to the 2^+ state, which is then de-excited by inelastic scattering in the exit channel

This complicated calculation can be carried out without any adjustment of parameters The wave function of the two transferred protons to be added to the target was obtained using the standard Bardeen–Cooper–Schreiffer method with the Random Phase Approximation The single-particle energies were taken from previous work and the strength of the pairing interaction was determined by fitting the experimental separation energy The separation of the two protons from the ^{16}O was calculated using the fractional parentage coefficients of Cohen and Kurath

The results of this calculation, taking the two-step processes in the exit channel into account, are shown by the full line in the figure The agreement is excellent, indicating that the essential physics of the process is now well understood In particular, it is clear why the inclusion of the two-step processes produces such a dramatic improvement Since the ^{78}Se and ^{76}Ge nuclei are both superconducting, the transition from the ground state of the target to the ground state of the final nucleus is much stronger than the transition to the first excited state Thus inclusion of the two-step process greatly enhances the 2^+ cross section but has a much smaller, though still important, effect on the reaction to the ground state

There are still more effects that could be taken into account, in particular the inelastic effects in the entrance channel In the calculation of the form factor of the transferred protons only the most important term, corresponding to 0s relative motion of the two protons, was taken into account, and a fuller calculation could investigate the contribution of the higher order terms Nevertheless, the success of the present calculations suggests that the most important effects have already been included, and that these improvements will not substantially affect the detailed understanding of this reaction that has now been achieved

Gels and chains

from a Correspondent

IT has been known, since the work of the Singers and of Allison in the 1950s, that gel formation in solutions of deoxy sickle-cell haemoglobin (Hb–S) is highly dependent on Hb–S concentration and that the minimum gelling concentration (MGC) is a function of temperature, *p*H, ionic strength and the presence of organic phosphates which bind preferentially to deoxy β-chains More recent studies by Bookchin, Nagel and Ranney on mixtures of deoxy Hb–S with other haemoglobins showed that the MGC values were higher, but that the partial concentrations of Hb–S at gelation were lower, indicating the incorporation of other haemoglobin species in the gel Following an earlier analysis of these results by Minton on the basis of a two-step aggregation process, they have now been re-interpreted by Moffat (*Science*, **185**, 274, 1974), assuming the formation of hybrid tetramers formed by association of unlike dimers in such binary mixtures, for example $\alpha^{A}{}_{2}\beta^{A}\beta^{S}$ from $\alpha^{A}\beta^{A}$ and $\alpha^{A}\beta^{S}$ in a mixture of Hb–S and normal adult Hb–A

According to Moffat all possible tetramer species are in principle capable of incorporation into the gel the gel forms when the weighted sum of their concentrations reaches a critical value, the MGC for pure deoxy Hb–S under the same conditions, which is assigned a weight of unity. The weights (w) can be regarded as relative association coefficients for the addition of particular tetramer species, hybrid or symmetrical, and deoxy or liganded, to the haemoglobin polymer. Since, however, deoxy tetramers have extremely small dissociation rate constants, hybrid formation is rapid in mixtures in the oxy form (as used by Bookchin and coworkers), and subsequent deoxygenation effectively freezes the resulting equilibrium mixture of tetramer species. From the value of the identical dissociation constant found for separate and mixed oxy Hb–A and Hb–S, Moffat evaluated individual tetramer concentrations and hence derived weighting coefficients for the tetramer components of the mixtures under consideration. The structural implications of these weights are clearly complex, and no clearcut generalisations emerge. For deoxy foetal haemoglobin (Hb–F) w is about 0.1, a result of some clinical interest in view of the proportions of Hb–F found associated with Hb–S in some clinical conditions. The weights do not depend greatly on the ligation state of the non-S chains, for example deoxy Hb–A and cyanmet Hb–A both have weights of about 0.5. The high w values for hybrid tetramers are also noteworthy. From the w values for deoxy Hb–A and deoxy Hb–S Moffat predicted that mixing in the deoxy form, with no hybrids present, would result in a higher MGC than mixing in the oxy form, facilitating hybridisation, followed by deoxygenation. This prediction was confirmed experimentally over a wide range of fractional composition. Moffat regards gelation as a highly cooperative process in contrast to Minton, who envisaged a two-step model in which linear polymerisation of haemoglobin tetramers into filaments is followed by lateral aggregation of the filament to form the gel.

An explanation at the molecular level for the depression of α or β chain biosynthesis in the respective thalassaemic states must be looked for by the study of gene expression and protein synthesis. Some progress has been made in elucidating the molecular basis of two forms of α-thalassaemia. Clegg and Weatherall (*Lancet*, ii, 133, 1974) now suggest that increasing circumstantial evidence for the relative instability of the messenger RNAs coding for the δ-chain of human Hb–A$_2$, and for the various $\delta\beta$ (Lepore) and $\beta\delta$ (anti-Lepore) chains, may be relevant to the molecular defect in some β-thalassaemias. Both Nance and Stamatoyannopoulos and coworkers have previously considered the possible role of fusion genes, of the Lepore type, in β-thalassaemia. Clegg and Weatherall now suggest that B^0 thalassaemia, in which there seems to be total absence of β chains, may be associated with a δ-like pattern of β-chain synthesis. In Nance's model this would involve silent Lepore-type fusion genes coding for normal β-chains, but controlled like a δ-chain gene. Clegg and Weatherall suggest that the instability of δ-chain mRNA is due to loss of stabilising regions at the 5′ and 3′ ends, subsequent to the evolutionary divergence of the β and δ chain genes. They discuss possible tests of their hypothesis, one of which would be the detection of small proportions, say 1%, of Hb–A in untransfused homozygotes for β-thalassaemia, containing normal β-chains but formed only in early nucleated erythroid precursors by the unstable mRNA.

Photographing a superfluid vortex

from P. V. E. McClintock

IT has long been realised that superfluid helium cannot rotate bodily: it is believed that, instead, rotation of the container may result in the formation of one or more individual quantised vortex lines. Particular interest therefore attaches to a report by Williams and Packard of the University of California at Berkeley in a recent issue of *Physical Review Letters* (**33**, 280, 1974) that they have been successful in obtaining photographs apparently showing for the first time the positions of individual vortex lines in superfluid ^{4}He.

The linear vortex, a classical phenomenon which was investigated in great detail by Lord Kelvin, consists of a cylindrical core which may or may not be hollow and, surrounding it, rotating fluid whose tangential velocity v_s is inversely proportional to the distance from the centre of the core. The vortex which forms above the plug hole as water flows out of a bath is a familiar example of a hollow cored vortex.

Vortices in superfluid helium are, however, of special interest because they are quantised: the quantity $\int v_s \, dl$, evaluated around any closed loop in the superfluid and known as the circulation, is always equal to $n(h/m)$ where h is Planck's constant, m is the mass of helium atom, and n may be zero (no vortex core present inside the loop) or a positive integer. The flow of superfluid around the core can be described by a macroscopic wave function and occurs without dissipation of energy, much like an electron in a stable state around the nucleus of an atom, so that the existence of quantised vortices may be regarded as a macroscopic manifestation of quantum mechanics.

Thus, when a container of superfluid, initially at rest, is rotated sufficiently slowly, the superfluid will always remain at rest relative to the fixed stars, no matter how long the experiment is continued. If the angular velocity is increased it will, however, eventually become energetically favourable for one or more vortex lines to be created in the liquid. At large rotational speeds many vortex lines are present and the resultant motion of the liquid approximates to solid body rotation at the same angular velocity as that of the container. It had been widely assumed that the vortex cores which in helium are approximately of atomic dimensions, would all be arranged parallel to the axis of rotation and spaced in a triangular lattice relative to each other. But despite extensive experimental evidence both for the existence of vortex lines and also for their quantisation, nobody had previously been able to determine experimentally their actual positions and orientations in the container, and it is this which Williams and Packard have now accomplished.

In essence their technique was extremely simple and exploited the fact that when free electrons are injected into liquid helium they form relatively large bubble-like structures of radius about 16 Å. These negative ions, by virtue of the reduction in energy arising from the excluded rotating superfluid, are very strongly attracted towards the core of a vortex. Thus, by using a radioactive electron source and a suitable arrangement of electric fields, it was possible to charge the array of vortices inside a rotating container of superfluid helium so that each vortex had negative ions spaced out at intervals along its core, rather like a string of beads. Then, by an appropriate reversal of electric field, the ions could all be shot off the vortex lines and out through the surface of the liquid. By positioning a scintillator above the surface it was possible to ensure that the current pulse arising from each vortex line would give rise to a spatially separate flash of light, thus indicating the position of that vortex in the liquid.

In practice, despite its apparent simplicity, the experiment has proved to be considerably more difficult than was the equivalent experiment to demonstrate the lattice of vortices in a superconductor. To achieve their successful result, the authors had to perform the not inconsiderable technical feat of mounting a complete ^{3}He–^{4}He dilution refrigerator, capable of reaching temperatures below 0.1 K, on a rotating table, it had been necessary to add a small proportion of ^{3}He to the superfluid ^{4}He in order to stabilise the vortex array, a magnetic field was required to focus the pulses of electrons leaving the liquid, and a very high quality optical system, using fibre optics to bring the feeble flashes of light up from the cold end to an image intensifier at room temperature, had to be developed The light from the image intensifier passed into a wide aperture camera loaded with high speed film

It was found that, with the apparatus in rotation, a series of spots did indeed appear on the film whereas, at rest, a completely blank negative resulted The density of spots was roughly equal to the expected density of vortex lines in the liquid, and was proportional to the angular velocity

It turned out, however, that the vortex lines were not in fact arranged in a regular manner, and moreover they appeared to wander around in the liquid in a random manner The reason for this is not yet clear but, now that a successful technique for photographing individual vortex lines has been developed, the topic will no doubt be the subject of further theoretical and experimental investigations

Control sites in the lactose operon

from Benjamin Lewin

THE lactose operon has been subjected to such intensive genetic analysis during the past decade that it is perhaps surprising that not all possible classes of mutant were isolated long ago That the mutants initially taken to identify the promoter in fact correspond to impairment in the action of the cyclic AMP system is, of course now well known The wild type lactose operon can be expressed at its full level only in the presence of the cyclic AMP receptor protein (CRP), activated by cyclic AMP The mutants previously isolated which reduce to 2% of its wild type maximum the expression of the lactose operon all map at the left end of the region between *lacI* and *lacO*, since they fail to recombine with the L1 deletion that covers this region, the reduction to 2% maximum expression is shown also by cells which contain a wild type lactose operon but lack a functional cyclic AMP system The far left end of the lactose control region therefore seems to provide a DNA site which is necessary for the interaction of CRP and RNA polymerase Mutants located between this site and the operator have been isolated which allow a high degree of operon expression in the absence of the cyclic AMP system, presumably by creating a site which allows the RNA polymerase alone to initiate transcription

The model accepted to explain these results supposes that the promoter of the lactose operon is a complex structure, its left end providing for an interaction with the CRP and its right end providing the site where RNA polymerase binds and initiates transcription How the interactions at these sites are related is not clear A prediction of this model is that mutants which reduce the level of expression of the operon by lowering the affinity for RNA polymerase of its binding site should map at the right end of the promoter region

The isolation of these mutants, constituting the first new mutant class to be reported for some time in the lactose operon, has now been accomplished by Hopkins (*J molec Biol*, **87**, 715–724, 1974) Certain repressor mutants share with promoter mutants the phenotype of reduction in maximum operon expression since they prevent induction, but the promoter mutants alone reduce the basal level of lactose enzymes in the uninduced cell Using this distinction to identify promoter mutants, Hopkins isolated two classes, the first comprise the previously identified kind of mutation at the left end of the promoter and show failure to respond to the cyclic AMP system, the second class, isolated for the first time, are located at the right end of the promoter region as shown by their ability to recombine with the L1 deletion, and have the properties expected of mutants altered in the interaction with RNA ploymerase—they show about 6% of wild type expression in cells with an active cyclic AMP system and this level is reduced to less than 0 5% when the cyclic AMP system is inactive (wild type operons show 2% expression in the absence of the cyclic AMP system) All three mutations of this type lie between the right end of L1 and the operator The isolation of these mutants opens the possibility of analysis *in vitro* to determine exactly which stage of action of RNA polymerase is inhibited, clearly the next step in identifying the action of this part of the promoter

The binding of repressor protein to the lactose operon, hitherto thought to implicate only the operator site immediately to the left of the *lacZ* structural gene also may involve more than one site Using a competitive binding assay for repressor, in which the DNA to be tested is mixed with repressor in the presence of a standard (labelled) operon and the reduction in retention of label on filters is measured, Reznikoff, Winter and Hurley (*Proc natn Acad Sci USA*, **71**, 2314–2318, 1974) confirm that deletions of the lactose operon have no affinity for repressor and constitutive operator mutants have a reduced affinity depending on the mutation Promoter mutants show no reduction in repressor binding, including one deletion which appears to remove virtually all the promotor though leaving the operator intact, the independence of these two sites contrasts strongly, of course, with the overlap between them which is seen in phage lambda A deletion which removes *lacZ* but does not extend into the operator has the same affinity for repressor as wild type lac DNA, confirming that the operator does not extend into the structural gene

But the strong *lacO*^{c} mutant DNAs have a much greater affinity for repressor *in vitro* than would be expected from the effect of these mutations in the cell, two mutants which reduce the induction of lactose enzymes in the cell by 100 and 300 times show a reduction in affinity for lactose repressor of only 25 or 30 times And deletions which remove a large part of the operator show the same affinity for repressor as the operator-constitutive point mutations This suggests that some common residual binding of repressor might take place at a site present in all these mutant DNAs When these deletions extend into the first part of the *lacZ* gene, the residual binding remains, but when they extend further, the affinity for repressor is much lowered This suggests that a secondary binding site is present in the *lacZ* gene, some distance from the operator but within the first thousand bases

Since the operator seems to be at least 16 bases long, it is very unlikely that this second binding site might be the result of a coincidence of sequence, the probability of independent origin of a sequence with an affinity for repressor as great as 3-4% of that of the operator is low—some *lacO*^{c} point mutations might well show this order of binding No obvious role can yet be seen for this second binding site, although it is possible that it might act as a failsafe signal for blocking progress of polymerases that have passed the operator or that it might interact in some secondary structure with the primary operator, there is, however, some evidence from earlier work to argue against both these possible models The evolutionary origin of this site is an interesting question, some light on which may be cast by sequence studies of the *lacZ* gene as well as the control region If the second site does play some part in operon function, then its nucleotide sequence must be under two sets of selective constraints, one concerned with its binding of repressor and one with the amino acid sequence specified in β-galactosidase

articles

Radiation effects in the fusion power programme

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As the prospect of a commercial fusion reactor becomes more real, concerted research is needed into the damage that is likely to be caused by radiation during its lifetime

In the past few years, our confidence that we can produce power from controlled thermonuclear fusion has grown, while we have also become more aware of the benefits of fusion power with respect to economy of resources and relative levels of pollution[1,2] As a result, the already large international fusion research effort is about to take another step and we are beginning to study the more practical aspects of economics and the detailed technological needs of controlled thermonuclear reactors (CTRs) This new character of the research is prompted by the high measure of confidence among plasma physicists that the necessary levels of plasma temperature, confinement time and density can be achieved so that a net power gain is achieved from fusion reactions Without fully understanding the plasma behaviour, they believe that, by scaling the plasma confinement system up in size, the conditions for the thermonuclear fusion can be achieved The choice of reactant will, however, be limited initially to the well known DT system

$$D+T\rightarrow {}^{4}\mathrm{He}\ (3\ 5\ \mathrm{MeV})+n\ (14\ 1\ \mathrm{MeV})$$

Since tritium is not available naturally, it must be bred within the reactor and the only likely reactions available are those of neutrons with lithium

With these two basic features in mind, some design features of a suitable CTR can already be discerned Studies of several different forms are under way[3,4] and preparations are being made to test the feasibility of large scale power production, with the aim of proving the case by the end of the decade[2] Given a positive answer, then the fusion programme would shift to a phase of developing large, efficient CTRs Those carrying out design studies are unanimous that we will need to build up a considerable body of new technological skills in order to build such a machine This article describes the research work which is going on in one particular facet of those skills—radiation effects—and, by outlining the extent of the radiation problems in the CTR, attempts to justify the contention that a lot of research and development remains to be done Similar treatments could be developed for other areas of CTR technology, a basic list of which is given in Table 1

Much of the fusion reactor technology can be discussed independently of the form of confinement used, and this is particularly true of the extensive damage and excitation effects which will occur in the confinement system surrounding the reacting plasma and the basic approaches to understanding and dealing with them We know, for example, that most of the fusion energy released will reside in the neutrons and that, as a result, the materials of the confinement vessel will be severely damaged by these neutrons and accompanying ionising radiation, while the levels of mechanical stress and temperature will also be high Radiation and other stress levels have been worked out for some reference designs and I shall draw heavily on the data for two of these, namely the United Kingdom Tokamak design and the Reference Theta-Pinch Reactor (RTPR) designed in the United States[5]

This arbitrarily chosen pair of reactors represent two divergent approaches to a power-producing toroidal reactor of which the engineering design is quite well developed Figure 1*b* shows a cross section of the RTPR and demonstrates the complexity of structure contemplated even in the concept stage It real life, the simple concentric layers will, of course, be interrupted by ducts and cables leading out from the plasma The coils for this design are not superconducting Figure 1*a* shows a segment of the United Kingdom Tokamak, with some speculative additions by myself to the concept of Mitchell and George[6] The neutron fluence values noted are not speculative, however, but derive from the neutronics and shielding calculations of McCracken and Blow[7], however, they are 'worst case' values for a first-wall energy flux value of 10 MW m^{-2} when, in fact, a wall loading value one tenth of this may be adopted It should be noted that, in both designs, a considerable neutron fluence emerges from the toroid and, if equipment is to be placed within the biological shield, a wide variety of electronic components could receive significant irradiation[8]

Figure 2 shows calculations of the irradiation rates for the two reactors[7,9] Gamma rays are generated by interaction of the neutrons with the structural materials To the materials scientist, these radiation levels seem unpleasantly large, although not much greater than those experienced near the core of a fission reactor In the CTR, however, the demands on components and materials are greater in that a high-vacuum chamber, powerful magnets and numerous other complicated systems have to be operated in this environment, as well as the coolant system The presence of a wider variety of high performance components is likely to compound the problem considerably Before discussing these specific effects, however, I shall make some general remarks concerning radiation effects at the high levels expected

Table 1 Major elements of fusion reactor technology

Physics of plasma confinement
Design of breeding blanket
Design of heat transfer system
Design of superconducting magnets
  (magnetic confinement)
Design of lasers
  (inertial confinement)
Design of tritium extraction system
Design of fuel injection system
Design of plasma exhaust system
Design for environmental effects
  (radiation, temperature and so on)

Damage phenomena

The permanent effects of radiation can be divided into two main classes damage distributed throughout a material ('bulk damage') and damage localised near surfaces ('surface effects') Under neutron bombardment, three forms of bulk damage are important—atom displacement, gas generation and the generation of solid transmutation products The degree of bulk damage is still significant in the region outside the breeding blanket, say at a neutron fluence of 10^{18} cm^{-2} Ductility, creep and stress-rupture/fatigue resistance, as well as the volume of metals, may all be modified and, in some metals, a sharp ductile-to-brittle transition occurs at a fluence of about 10^{20} cm^{-2} at room temperature Formation of gas bubbles may follow from gas generation and produce changes in mechanical properties by virtue of the accumulation of bubbles at grain boundaries[4] Void formation, a product of very extensive atom displacement[10] produces both swelling and a catastrophic loss of strength The fluence at which this effect sets in varies considerably with the type of metal, purity and temperature, varying from 5×10^{19} to 10^{22} cm^{-2} Even at 10^{18} cm^{-2}, before voids appear, the formation of other microstructures, such as interstitial loops and precipitates of supersaturated constituents, affects mechanical strength

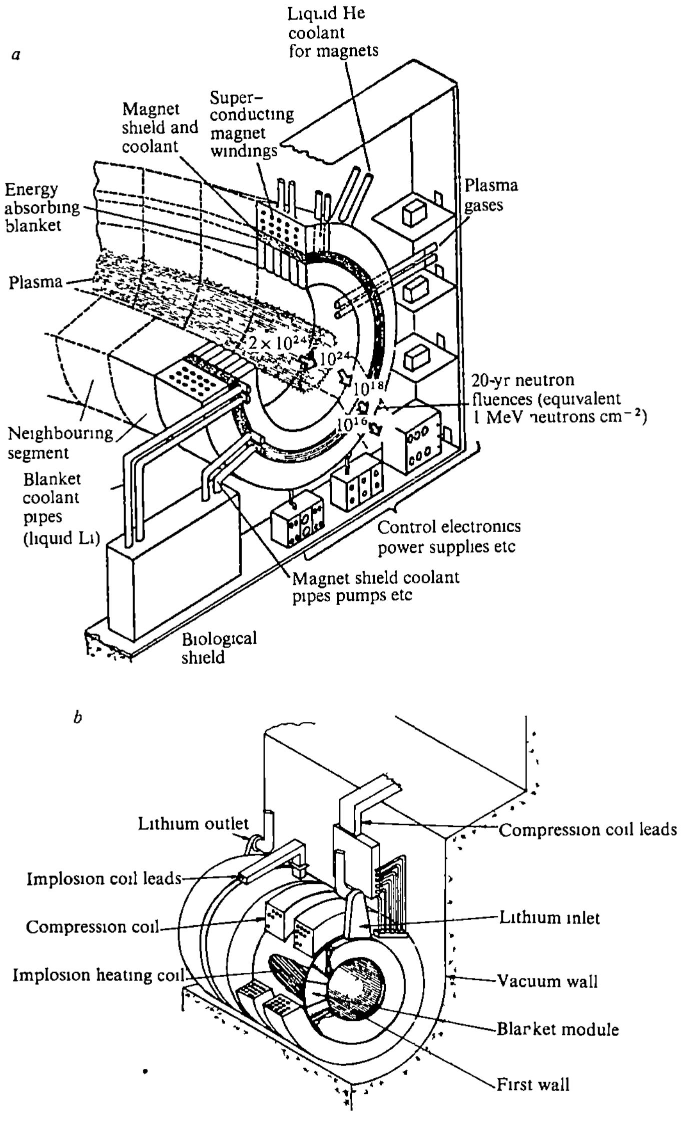
a
Liquid He coolant for magnets
Magnet shield and coolant
Superconducting magnet windings
Energy absorbing blanket
Plasma
Plasma gases
2 x 10^24
10^24
10^18
10^16
20-yr neutron fluences (equivalent 1 MeV neutrons cm^-2)
Neighbouring segment
Blanket coolant pipes (liquid Li)
Control electronics power supplies etc
Magnet shield coolant pipes pumps etc
Biological shield
b
Lithium outlet
Implosion coil leads
Compression coil
Implosion heating coil
Compression coil leads
Lithium inlet
Vacuum wall
Blanket module
First wall

Fig 1*a*, Segment of the United Kingdom Tokamak fusion reactor showing calculated neutron fluences for a life of 20 yr Structural design by Mitchell and George[6] External equipment shown may have to be placed outside the biological shield Outside diameter of segment is 4 m *b*, Cross section of the Reference Theta-Pinch Reactor (RTPR) showing functional regions of the toroid[5] Outside diameter of segment is 1 5 m

The resistivity of a metal is increased by the formation of defects, which act to scatter the current carriers At a fluence of 10^{17} cm^{-2} the resistivity of copper has increased by a few percent and, at 10^{19} cm^{-2}, by a factor of 4 (ref 11) In superconductors, the critical temperature for superconductivity and the current density are altered slightly by neutron fluences in the 10^{18} cm^{-2} range

Surface effects will be significant at those free surfaces of metals which are exposed to very high energy fluxes Neutron energy imparted to the lattice can cause sputtering and gas bubbles generated near surfaces can produce blisters which may then burst The first wall is the most heavily bombarded surface and the special surface effects expected here will be described later

The insulators in a CTR will have a less obvious but still demanding role Although, in general, metal parts will have been designed for a single role (for example, mechanical support or electrical conduction), insulators often have to fulfil combined mechanical and electrical roles and must therefore be more resistant to change To take a simple example, a high voltage feed-through insulator has both to support a conductor and to act as a barrier to conduction This electronic inactivity, however, is not easy to maintain when the material is being heavily excited by nuclear radiation at a high temperature The wide-band dielectrics are no longer insulators under these conditions (see, for example ref 12)

To emphasise the general and fundamental nature of these changes it should be pointed out that even at neutron fluence levels of the order of 10^{19} cm^{-2}, crystallinity is destroyed in many inorganic dielectrics at room temperature[13] and insulators, which are usually transparent in the visible and near ultraviolet, may, at this level of bombardment be opaque because of defect absorptions Likewise, organic materials, which rely on very specific molecular structures would, under these circumstances, receive sufficient energy to break every chemical bond several times Thus, unlike a metal, the insulator may undergo a partial breakdown of the main quantum mechanical features which determine its technological use—its low conductivity and wide band gap

Specific problems

Two well defined functional regions of the toroidal fusion reactor stand out as damage problems—the breeding blanket and the containment magnets In the first, the energy flux is very large and will include many species other than neutrons (α particles, γ rays, soft X rays, energetic ions, thermal plasma and so on), in the second, a critical level of performance has to be maintained Further, throughout the reactor, at points yet to be determined, there will be electrical and electromechanical components which must operate reliably, such as valves, relays and cables I shall deal with the main functional regions first

The first wall will be a thin metal barrier between the reactor vacuum and molten lithium coolant The most commonly quoted metal for the wall is a niobium alloy but other refractory metals (V, Mo) and several other groups of metals are being considered, for example austenitic stainless steels, nickel alloys, sintered aluminium powder, silicon carbide and graphite There have been no tests on these metals with 14 MeV neutrons at high temperatures and fluences because of the difficulty of generating intense neutron beams of this energy, but irradiations in high flux reactors (neutron energies in the range 0 1–5 MeV have been carried out on many stainless steels and the information has been supplemented by gas implantation experiments[10] Major stresses on the first wall will, however, be the repeated thermal shocks as the reaction is started and stopped cyclically, and the high stresses created by the

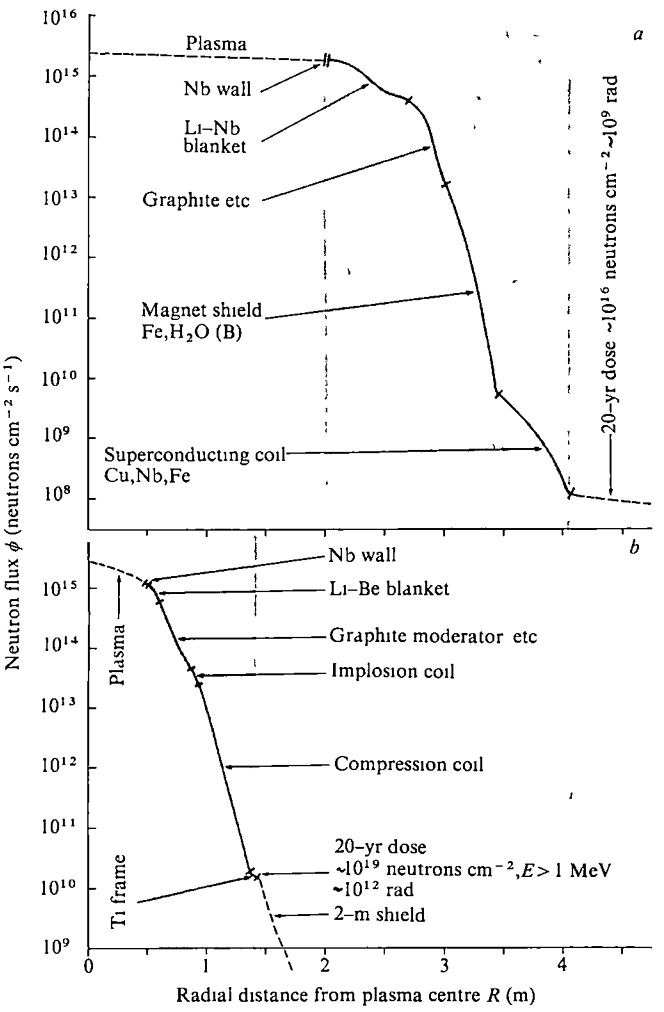

Fig 2 Radiation levels in (a) Tokamak[7] (10 MW cm^{-2}) and (b) RTPR[5] (6 7 MW cm^{-2}) showing larger size and heavier shielding in Tokamak Shading indicates functional structures

magnetic confinement field Such a cyclical mode of operation will vary in severity between the theta pinch and "DC" type of reactor but, in both cases, cyclical stresses will be much greater than have been experienced in fission power reactors, which are operated in a 'steady state' Unfortunately, theories of fatigue failure are not well advanced and thus it is fair to say that we do not yet know whether first wall can be fabricated which will take the stresses required at an economic size Mitchell and Booth, however, have evolved a flexible design of helium-cooled cell for the first wall which will allow the designer to minimise the mechanical forces acting on the wall[14]

The main surface effects which have to be considered for the first wall are

(a) thermal evaporation caused by the high energy flux,
(b) sputtering by the plasma ions, which will include deuterium, tritium, helium and some heavier ions originating in the wall,
(c) blistering arising from gas implantation,
(d) neutron sputtering

These effects are doubly deleterious to reactor operation since they result both in erosion and the introduction of impurities into the plasma The flux, φ, of ions from the plasma can be estimated using the expression

$$\varphi=[5\ 6\times10^{13}\ W/F]\ \mathrm{cm}^{-2}\ \mathrm{s}^{-1}$$

where W is the wall loading in MW m^{-2} and F is the fraction of ions which undergo fusion Sputtering yields are only known to within a factor of 2 to 3 for light ions[15] and the erosion rates of neutron sputtering and blistering effects are not yet established, since the phenomena have only recently been discovered Blistering effects vary with ion energy, target temperature and dose rate[16], apart from the spalling of the blistered surface, the blisters also act as thermal impedance and should lead to enhanced surface evaporation of the first wall Neutron sputtering was thought to be negligible[15] but Kaminsky and coworkers[17] have recently found rates to be higher than for light ions (as high as 0 25 atoms per neutron), which could lead to intolerable erosion rates

A major problem in the design of the theta-pinch type of reactor is that the first wall must be coated with an insulator to withstand the transient electrical field generated when the plasma is made to contract or implode[4] Operating at a temperature of over 600° C, it must withstand a field of about 10^7 V m^{-1} for about 1 μs When the plasma ignites, the insulator will be exposed to a thermal shock and a burst of particles Although Bunch and coworkers[9] feel that some forms of alumina ceramic may have the intrinsic mechanical strength to withstand the thermal effects, it is not yet known whether such a material will maintain its dielectric strength or integrity under the multiple particle and photon bombardment effects which will accumulate Table 2 lists some of these effects It is worth noting that the peak ionising dose rate in this insulator (A G H - S , unpublished) is likely to be greater than a rate which could lead to the transport of amperes of current per square metre of surface, and the material cannot be considered as insulating at all times

Superconducting magnets face both a radiation-heating problem and a radiation-damage problem The energy flux of neutrons at the magnet can cause excessive heating unless shielding is added within the confinement toroid The Tokamak design incorporates such a secondary shield of water-cooled steel McCracken and Blow[7] have made a detailed study of the neutron-induced changes in aluminium and copper, two of the materials which may be used as conducting matrices for superconductive filaments, these authors conclude that the neutron fluence over 20 yr can be allowed to rise to 10^{18} cm^{-2} if the magnets are allowed occasional annealing treatments, at room temperature or above Copper has advantages in strength over aluminium, and neutron irradiation actually improves the yield stress levels in copper, although more creep than usual occurs under neutron irradiation because of the enhanced atom motion Since the mechanical forces on conductors and the structure of powerful magnets are very high, creep effects may be significant Conductivity degradation is also one of the more difficult effects to simulate—mechanical forces have to be applied to metal and dielectric samples at high neutron fluxes and extremes of temperature At the moment test sources with the required neutron fluxes (about 10^{15} cm^{-2} s^{-1}) are not yet available and research may have to be started using fission neutrons and ion beams which can give the correct knock-on energies although only over limited volumes of material

In the theta-pinch design, the compression coils are not superconducting and no special measures are taken to reduce neutron heating Neutron fluxes will be more than four orders of magnitude higher but some of the damage will anneal as it is formed

The insulation of the magnet coil conductors presents more of a problem in both designs At present, thin organic layers are placed between each conductor, with organic potting to give the very necessary binding support The total radiation doses at the coil are probably high enough to destroy the mechanical properties of most organic insulators, and inorganic insulators will probably have to be used In the latter materials, displacement and transmutation can permanently increase conductivity and reduce breakdown strength, internal buildup of space charge can lead to very high internal fields and spontaneous breakdown

Table 2 Fusion reactor technology—summary of possible radiation effects problems in insulators

Physical phenomenon	Major functional effect	Class of components strongly affected	Basic studies (if any) needed	Possible technological solutions
(1) Electrical effects				
Generation of displacement defects leading to				
—Changes in carrier mobility* —Generation of defect levels	Changes in conductivity Changes in dielectric loss Changes in dielectric constant	First wall Standoff insulators Capacitors	Effects of defects on carrier transport Radiation induced defect production	Development of special neutron-tolerant glasses or ceramics with special doping grain size, and microstructure
Amorphisation leading to				
—Loss of directional properties	Piezoelectric device failure	Oscillators Acoustic devices	Physics of amorphous solids and amorphisation	Development of methods for depositing neutron-tolerant films of insulators
—Band-tailing and so on			Electrical breakdown	
Transmutation, precipitation of products	Changes in dielectric breakdown strength at precipitates and pores	All insulators	Precipitation of transmutation products	
Space-charge build-up	Internal breakdown Unwanted potentials		Charge traps in Insulators	
Photoconduction	Transient conductivity		Photoconductivity in insulators and interfaces	Insulators of optimised conductivity under irradiation (controlled leakage)
Changes in secondary emission and photoemission	Changes in first-wall emission efficiency		Surface barriers in solids	
(2) Optical effects				
Defect production	Changes in	First wall	Colour centres	Passivated, non-darkening surface coatings
	(a) transmission and reflectance	Windows	Emissivity	
Surface loss of stoichiometry	(b) absorptivity/emissivity relations controlling equilibrium with plasma	Light guides	Surface radiation damage	Radiation-tolerant light guides
			Surface reactivity	
(3) Thermal effects				
Colour-centre production	Decrease in radiative heat transfer	First wall	Colour centres	Dielectrics of high thermal conductivity
Lattice disruption and impurity precipitation	Decrease in heat transfer by way of lattice	Lagging of coolant pipes Superconductor insulation	Lattice vibrational modes	
(4) Mechanical effects				
Thermal shock Radiation-induced swelling (anisotropic) from defect formation	Fatigue fracture Microcracking Debonding Loss of strength	All dielectrics, especially first-wall coating, spacers and coil insulators	Microstructure and strength of materials Generation and rates of annealing of point defects and voids	Use of isotropically expanding dielectrics (e g glasses), Use of creep to relieve swelling Optimisation of powder size in ceramics Insulators with (a) Rapidly annealing defects (b) low void formation Special solid lubricants
Enhanced atom motion	Creep	Precision parts	Atomic diffusion under irradiation	
Phase changes and so on	Increase of friction Loss of directional properties and strength	Valves	Friction and microstructure	
		Piezoelectric devices	Radiation-induced phase changes	
Sputtering	Erosion of thin layers	First-wall coatings	Sputtering physics	
(5) Chemical effects				
Reduction by hot hydrogen	Reduction of oxides, nitrides etc	First wall Other coatings	Reactions of atomic hydrogen at surfaces	Passivated coatings
Diffusion of, for example, oxygen from insulators	Deoxygenation and so on of oxide insulators by Li, Nb and so on	First wall Oxide/Nb/Li system	Oxygen diffusion in metals and insulators	Oxygen diffusion barriers

* Carrier mobility has a strong influence on radiation induced conductivity in insulators

effects Finally, the leakage currents arising from photoconductive effects cannot be ignored In the case of the theta-pinch coil design, the coil insulators may experience dose rates in the region of 10^6 rad s^{-1} and photoconductivity may cause the resistivity of inorganic insulators to change from a normal 10^{18} to 10^{10} ohm cm during the pulse, leading to leakages in the ampere range Also, the use of implosion heating requires the application of a fast pulse at a voltage of more than 100 kV at the coil terminals, local fields could thus exceed the dielectric breakdown strength in the irradiated insulators In the case of superconducting coils, fields are low in normal circumstances but if the superconducting-to-normal transition occurs, a voltage of up to 20 kV can appear across the terminals Doses in the well shielded Tokamak magnets, however, will probably be only $\sim 10^2$ rad s^{-1}

Proceeding from the plasma outwards, the first region in which electronic devices might be placed would be in the void between the coils and the biological shield Some control circuits and actuators for the coolant and vacuum pumping systems may need to be near the torus Radiation dose rates can be reduced by local shielding but a 20-yr zero-shield figure of 10^{16} neutrons cm^{-2} and 10^8 rad for this void region is not unreasonable to expect This is a level which requires a special selection of all semiconductor and dielectric parts (including organics) used in this region[8]

There remains a class of damage problems which cannot yet be described in detail but of which the outlines are clear The nuclear fuels, deuterium and tritium, must be passed into the reactor, probably by means of particle (pellet or ion) accelerators, currents must be passed through the plasma, impurities must be "diverted"[18], spent fuel must be pumped out, eddy currents must be suppressed Each of these functions requires the presence of a virtually independent subsystem—as yet of uncertain design—with elements near to the plasma The materials needed and the fluxes to which they will be exposed will become known as the detailed geometry of reactors is developed (see, for example ref 19) but it is not too early to develop a general knowledge of the effects of high fluxes of neutron on those types of metal which may be used and of combined neutron–γ effects on the dielectrics Some idea of the materials of interest can be gained by examining the materials commonly used in various component technologies (see, for example refs 8 and 20) A case in point is the window problem in the laser fusion reactor (see for example, paper 8 of ref 4) Light transmission, reflectivity and dimensional stability are all key performance parameters in this precision optical system and, as Table 2 shows, all are affected strongly by high levels of neutron and γ irradiation (estimated doses are $2\times10^{20}\ \mathrm{cm^{-2}\ yr^{-1}}$ and $10^{11}\ \mathrm{rad\ yr^{-1}}$, respectively)

Basic research needed

This list of hazards for the CTR system need not make us drop the idea of building such a machine The research on radiation effects done in support of the fission reactor programme and the 'hardening' of semiconductor devices for aerospace use has shown that solutions can usually be found for each problem if an understanding of the phenomenon is first achieved, however, such solutions have often in the past proved to be highly individual and specific (see, for example, the beneficial use of lithium in silicon solar cells[21] which, however, does not benefit silicon transistors) and take a long time to put into effect Thus, if fusion technology is to proceed according to a proper timetable, a form of 'preventive research' must be undertaken in which the basic phenomena of radiation effects for any material of interest are studied to the point that any potentially crippling problems of degradation will have been noticed before the development of that material has proceeded unduly far[20]

For metals, some of the damage phenomena described in the earlier sections are already being studied as part of fast fission reactor development, but these must be widened to include surface effects and to embrace a wider range of metals For dielectrics, Table 2 provides a comprehensive list of damage phenomena In looking for neutron-tolerant dielectrics, the glasses are of special interest because they are not prone to the anisotropic expansion which can lead to loss of strength in crystalline insulators[22] The fission reactor programme has already demonstrated the tolerance of certain refractory oxides and other ceramics to radiation It is assumed that semiconductors will be virtually excluded from the high flux regions[8]

In the field of theory, recommendations have been made for development in theoretical models for creep, embrittlement, crack formation and void formation, especially in the refractory metals[23], for dielectrics, models will be required for damage to electrical and optical properties as well as to mechanical properties Up to now, the electrical and optical properties of insulators have not been of central importance in the nuclear energy field and as a result, there is a smaller body of damage theory on which to base future work than for metals Furthermore, the technologically important oxides (such as silica, alumina and zirconia) are crystallographically complex and for this reason have not received the required degree of attention from theoreticians as media for band theory and defect studies

Aims and timetable

Having stated the topics for basic research, I shall briefly lay out a set of technological aims for dealing with radiation effects in CTR components, based on a timetable for general CTR development by Hancox[24], as follows

Metals. (1) Development of structural metals with minimum change in ductility, creep, stress rupture, fatigue and swelling under high neutron fluences at high temperatures (and with high resistance to corrosion by the breeding material) (2) Development of conductor/superconductor with the minimum change in resistivity and creep strength at very low temperatures and moderate neutron fluences (3) Control of surface erosion phenomena such as blistering, sputtering and gas desorption

Insulators. (1) Development of structural ceramics and glasses and a range of electronically passive thin coatings resistant to radiation-induced swelling, cracking, leakage, high voltage breakdown and chemical attack (2) Development of special deposition methods for insulating coatings (3) Development of radiation tolerant light-handling media (4) Development of dielectric materials to perform special electronic functions under high neutron-γ ionisation

Taking 1979–84 as the period during which feasibility of fusion reactors will be tested, we can also draw up a tentative timetable giving a concerted fundamental and technological development and which will permit the successful construction of short lived experimental reactors by 1979 and long lived, economic prototype reactors in the early 1980s, as follows

1974–79: Preparatory research. Fundamental research on damage and structure of reactor materials, especially dimensional stability of metals and electronic stability of dielectrics, initial development of specific radiation-tolerant structures (especially electro-mechanical or electro-optical devices) required for the demonstration power reactor to be designed in the 1980–82 period, Simulation of neutron-γ, ultraviolet and plasma effects in metals and insulators using electron, ion and proton bombardment and the highest fluxes of non-fusion neutrons available, experiments on lower levels of neutron fluxes from early fusion plasma experiments (as available) for comparison with non-fusion sources

1979–82· Feasibility testing Fabrication of prototype radiation-tolerant materials and devices for a demonstration reactor, exposure of samples in feasibility-test reactor, full design of test rigs for demonstration reactor

1982–90: Demonstration reactor. Fabrication of radiation-tolerant materials and devices for demonstration reactor, accelerated life tests for 20-yr operation of prototype reactor, development of second generation of radiation tolerant materials and devices for a possible prototype commercial CTR

Conclusions

This review of one branch of fusion reactor technology has been made with the idea of distinguishing problems in radiation effects which deserve to be investigated during the next few years, even before reactor designs are finalised The field of insulator study has perhaps been given greater emphasis than that of metals because the latter has been dealt with at length in recent conferences and workshops while the latter has been somewhat neglected by comparison, thus, the multiplicity of effects produced in materials with a wide band gap has been stressed It seems, however that the strength of metals under irradiation may be the limiting factor in the life and efficiency of controlled thermonuclear reactors and thus an extensive programme of research and testing of metals should continue for the rest of this decade For both insulators and metals, techniques of predicting and dealing with radiation effects will need to be improved and 'preventive research' carried out on many of the less obvious materials The intelligent direction of resarch may well help to achieve the target of useful fusion power in the national grid not long after the beginning of the next century

I acknowledge the liberal assistance of Dr G McCracken in supplying details and comment on the United Kingdom Tokamak, making constructive comments on the manuscript and allowing the use of some unpublished text material F W Clinard, jun, of Los Alamos Scientific Laboratory made some useful comments on Table 2 in proof in a recent discussion

Note added in proof For Tokamak reactors it may be required that the torus as a whole must not constitute a closed conducting loop A completely insulating segment must thus be inserted in the torus, the inner edge of which will be exposed to the plasma This segment or "spacer" will have to maintain strength, dimensional stability and electrical properties over the range of radiation doses shown in Fig 2*a*

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Evidence for the breakup of eastern Gondwanaland by the early Cretaceous

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An analysis of early Cretaceous marine magnetic anomalies of south-western Australia helps to piece together eastern Gondwanaland It is suggested that India once abutted Western Australia

THE seafloor spreading history of the portion of the Indian Ocean basin between the Ninetyeast Ridge and Australia, the Wharton Basin, is poorly understood Most Gondwanaland reconstructions have placed the eastern coast of peninsular India against Antarctica, generally leaving a gap of about 15° of longitude between India and Australia[1-5] Some workers have placed the eastern coast of India against Western Australia[6-8] and yet others have favoured placing Indonesia, south-east Asia, even China, against Western Australia[9-11] I present here new evidence pertaining to the reconstruction of eastern Gondwanaland, the recognition of early Cretaceous marine magnetic anomalies adjacent to south-western Australia

Anomalies M-1 to M-11 inclusive of Larson and Pitman[12] have been identified between the northern edge of the Naturaliste Plateau and the Australian mainland The lineations, referred to here as the 'Perth sequence', vary in strike from 040° near the plateau to 020° near the mainland, they demonstrate that the age of the oceanic crust increases toward the continent (Fig 1) Although somewhat older crust may exist beneath the continental margin, the presence of anomaly M-11 dates the separation of India (?) and Australia in this region at 127 Myr BP, or earlier This date is nearly coincident with that determined for the opening of the South Atlantic Ocean[13]

Larson *et al*[14], suggested that the time scale of Larson and Pitman[12] should be compressed by 10–15% relative to anomaly M-22 A 10% compression has been used here

Anomalies M-2 and M-4, the most distinctive of the younger half of the M series, correlate well from track to track (Fig 2) The correlation of anomaly M-2 is dashed south of profile EL48 because tracks 1106-2 and EL54 cross onto the Naturaliste Plateau in this vicinity The model anomaly profile (Fig 2) uses a magnetic layer 500 m thick, with a remanent magnetisation intensity of 0 012 e m u cm^{-3} Models were also calculated for remanent magnetisation acquired at palaeolatitudes 50–70° S and for subsequent rotations of the plate of as much as 40°, both clockwise and anticlockwise The correspondence to the observed data, however, was not greatly affected The model profile used here (Fig 2) is for latitude 60° S and no subsequent rotation The correspondence between the observed anomalies and this model is comparable to that of the equivalent portions of the Cape basin sequence[13] and the Keathley sequence[15], which also exhibit relatively slow spreading rates

The identification of the M series is substantiated by Site 259 of the Deep Sea Drilling Project (DSDP), which gave

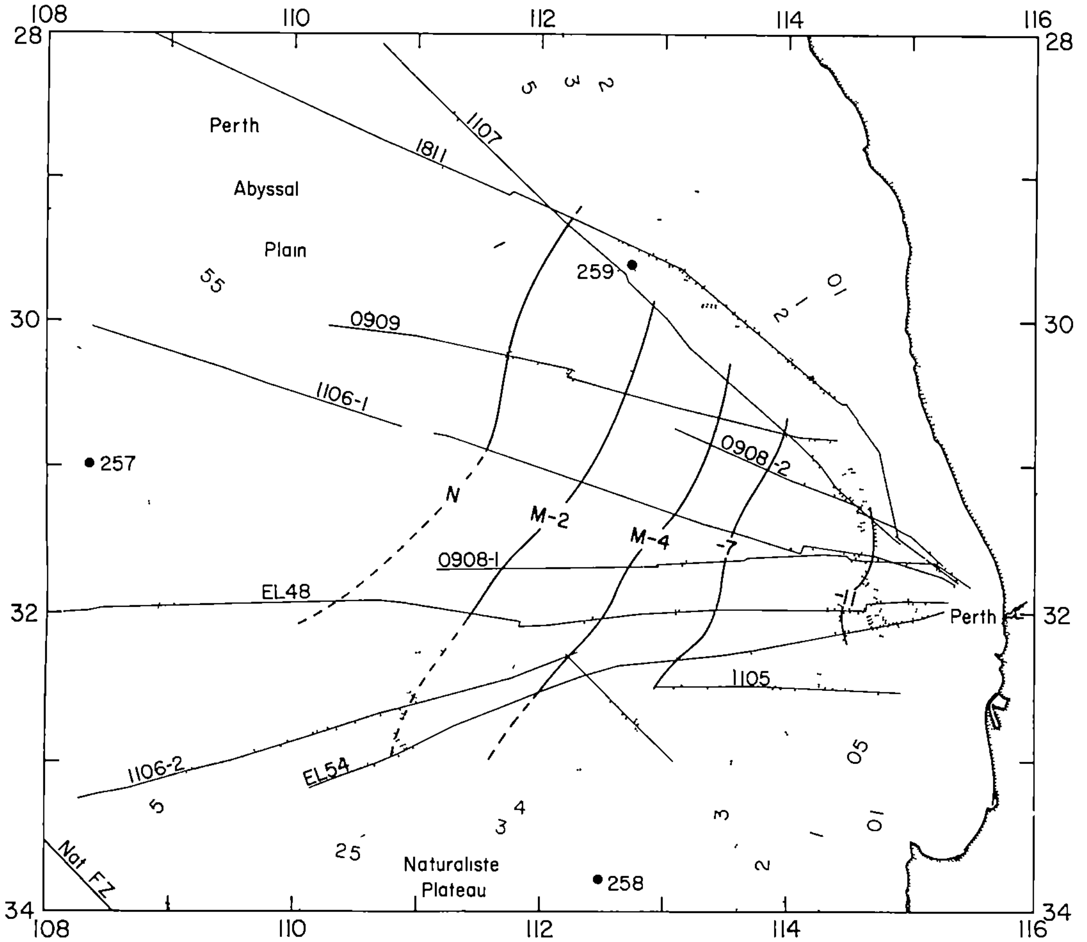

Fig 1 The Perth sequence of Mesozoic (M) magnetic lineations showing the portions of the ship tracks corresponding to the observed profiles shown in Fig 2 ●, DSDP site numbers, N, negative magnetic anomaly The bathymetric contours are in corrected metres

a basal sediment age of greater than 110 Myr (ref 16) Calculations based on anomalies M-2 (116 Myr BP) and M-11 (127 Myr BP) indicate that the (half) spreading rate during this period was 2 3 cm yr^{-1} Site 257, located in the late Cretaceous 'quiet zone'[17], yielded an age from near basal sediments of 102 Myr (ref 18), which corresponds to a (half) spreading rate of 2 1 cm yr^{-1} between Site 257 and the M-2 lineation

A negative anomaly just west of (younger than) the Perth sequence has also been correlated (Figs 1 and 2) This anomaly is probably equivalent to a negative anomaly adjacent to the Cape basin sequence which may represent a magnetic reversal[13]

Sclater and Fisher[11] identified east–west trending lineations (Fig 3) which indicate that the age of the crust of the northern Wharton Basin increases towards the south The assumption that this spreading regime also created the crust of the southern Wharton Basin led them to conclude that India could not have abutted Australia in Gondwanaland

The Perth sequence, although oriented at about 55° to the younger lineations of Sclater and Fisher[11], was initially considered to be related probably to some segment of their anomaly pattern, perhaps to the sliver bounded by their proposed 96° E and Investigator fracture zones (Fig 3) The intervening 20° of latitude between anomaly 33b (ref 11) and anomaly M-1 would represent the late Cretaceous (115–85 Myr BP) magnetic quiet period This scheme for the origin of the Perth sequence would require an average (half) spreading rate of more than 7 cm yr^{-1} during the quiet period, and a shift of the pole of rotation commensurate with the required change from NW–SE to N–S spreading The (half) spreading rate between DSDP Site 212 (approximately 95 Myr BP) (ref 19), which is 330 km south of anomaly 33b (Fig 3), and Site 256 (basal sediment age 101 Myr) (ref 18), is about 8 cm yr^{-1} However, the fact that Site 257 is fully 1,100 km south-east of Site 256, has the same age as Site 256, is obviously inconsistent with this scheme The slow spreading rate of the Perth sequence (2 3 cm yr^{-1}) also implies that the achievement of a 7 cm yr^{-1} average spreading rate between anomalies M-1 and 33b is not realistic Additional magnetic evidence from the southern Wharton Basin offers an alternative mode of origin for the Perth sequence

The overall 'unquietness' of the supposed quiet zone in the southern Wharton Basin is disturbing Peak-to-peak amplitudes of up to 700 γ are common These anomalies seem to be unrelated to the basement morphology and in many instances they can be correlated from track to track The similar basement ages of DSDP Sites 256 and 257, combined with a varying degree of axial symmetry on magnetic profiles in the quiet zone between these sites, led to the recognition of anomalies believed to be M-1 to M-22 inclusive, between the northern extension of Broken Ridge (Fig 3) and Ninetyeast Ridge Anomalies M-2 and M-4 can be traced for more than 400 km, striking 040° (Fig 3) The identification of the older anomalies is, however, based upon only one track—Vema 18—(Fig 4) In Fig 4 this profile is shown with a model anomaly profile for remanent magnetisation acquired at 60°S and 0° declination The character of anomalies M-1 to M-11 inclusive and their correlation with the model, is similar to that of the anomalies of the Perth sequence, the correlation of the observed and model profiles between anomalies M-11 and M-22 is poor The spreading rate between anomalies M-2 and M-22 is 1 6 cm yr^{-1} A negative anomaly just east of anomaly M-2 has been correlated between tracks, but it is questionable whether this anomaly is equivalent to the negative anomaly west of the Perth sequence Site 255 lies 70 km south-east of the extrapolated trend of anomaly M-2 It is, however, located on top of Broken Ridge, and it terminates 200 m above the basement, in 80-Myr-old material[18] Therefore it

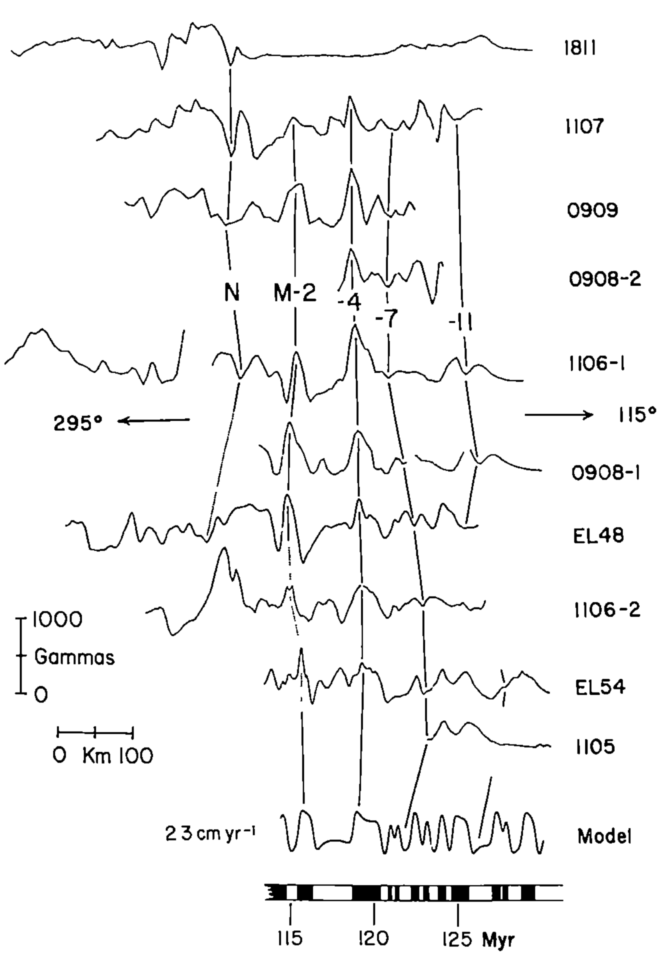

Fig 2 Observed magnetic profiles of the Perth sequence projected perpendicular to the strike of the lineations and plotted above the model anomaly profile, the latter was computed from the magnetic block model of Larson and Pitman[12]

probably has no potential as a support for the identification of the Broken Ridge sequence Lamont-Doherty has no data north of the Vema 18 track but profiles Lusiad-3 Project Magnet 541 (refs 11, 20) show anomalies which may represent M-2 and M-4 Along the LU-3 track the possible M-2 anomaly lies 155 km north-west of Site 256 (101 Myr BP), which would correspond to a (half) spreading rate of only 1 0 cm yr^{-1} The LU-3 data suggest that the lineations may curve toward the north (Fig 3) If the Project Magnet 541 data are also used, a 75 km sinistral offset of the lineations just north of the Vema 18 track is implied An offset here is not altogether unlikely, in view of the trend of a previously unreported fracture referred to here as the 'Naturaliste fracture zone' (Fig 3) The strike of this 650 km long fracture zone is well defined by seismic and bathymetric data in the vicinity of the Naturaliste Plateau

The axis of the spreading centre inferred to have generated the Perth and Broken Ridge sequences is not well delineated Figure 5 shows observed magnetic profiles from the quiet zone between the Perth and Broken Ridge sequences The corresponding track lines, as well as the trends of correlated anomalies, are shown in Fig 3 The magnetic trends consistently strike NE–SW to NNE–SSW, providing another argument against the probability of a direct relationship between the Sclater and Fisher pattern and the Perth sequence A prominent negative anomaly defines the axis of symmetry on profiles EL45 and 1105 (Fig 5) With the exception of the central anomaly, the large positive anomalies between C and C′ on profile 0909 are associated with large seamounts North of profile 0909 the position of the axis is based upon the overall symmetry of the magnetic profiles Bathymetric and seismic reflection data show that the northern half of the inferred axis of spreading coincides with the axis of a regional topographic and basement high The basement topography across the axis is generally smooth compared with active ridge crests although discrete seamounts are abundant in the axial zone between latitudes 28° and 29° S The positions of basement scarps which flank the axis are shown in Fig 3 The average thickness of sediment in the axial zone varies from 700 m at the southern end to 300 m in the north

Extrapolation of the spreading rates of the Perth and Broken Ridge sequences indicates that the inferred spreading centre would have become inactive by about 77 Myr BP The average basement depth in the axial zone is 5,275 m This depth compares favourably with the depth predicted (approximately 5,400 m) for a slow spreading ridge 77 Myr after the cessation of spreading[21]

There is a reasonable case for the existence of an extinct spreading axis in this region An approximate symmetry exists in the magnetic profiles along several tracks The axis of symmetry is essentially perpendicular to the trend of the Naturaliste fracture zone and parallel to those of the Perth and Broken Ridge sequence lineations The distance from the axis to these sequences is roughly proportional to their respective spreading rates, and it lies essentially midway between two holes of the same age (Sites 256 and 257)

Although the proposed late Jurassic–late Cretaceous spreading centre provides an explanation for the problem of the widely-spaced but similarly aged DSDP sites in the southern Wharton Basin[18 22], it bears problems of its own Anomaly M-11 lies adjacent to Australia and is the oldest anomaly of the Perth sequence The oldest identifiable anomaly of the poorly documented Broken Ridge sequence is, however, M-22, and there is room between M-22 and the Ninetyeast Ridge for still older crust This apparent discrepancy in the time of inception of spreading (as well as the apparent asymmetry of the spreading rates of the two sequences) may be alleviated by the fact that when the M-2 lineation (from Vema 18 southward) of the Broken Ridge sequence is rotated into alignment with the M-2 lineation of the Perth sequence, the former sequence is not juxtaposed with the latter Instead, the Broken Ridge sequence lies to the south-west, on the opposite side of the Naturaliste fracture zone This rotation of 13 4° about a pole at 40°N, 175°E leaves the Naturaliste fracture zone perpendicular to the lineations

Another difficulty which must be explained is the presence of the northern extension of Broken Ridge, which roughly parallels the magnetic lineations (Fig 3) Seismic profiles show that the northern end of this feature (delineated by the 3,000 m isobath) resembles the Naturaliste Plateau Although it is possible to fit these two plateaux together, the presence of the northern extension on crust which post-dates the inception of spreading (anomaly M-11) by 15–20 Myr precludes a simple rifted origin

As already stated, the spreading centre in the southern Wharton Basin might have become inactive by 77 Myr BP Anomaly 33 is not discernible in the axial zone, and as the magnetically quiet period is believed to have been marked by a pulse of rapid spreading[12], the cessation of spreading probably occurred earlier The presence of anomaly 33b in the northern Wharton Basin[11] indicates that the spreading centre which generated it was active by about 80 Myr BP No structural feature is known in the area between the two regimes (lat 20°–22°S) which could suggest the location of a possible boundary between them The magnetic anomalies in this area are typical of a quiet zone One way to reconcile these two spreading regimes is to postulate that the older spreading axis jumped to an east–west

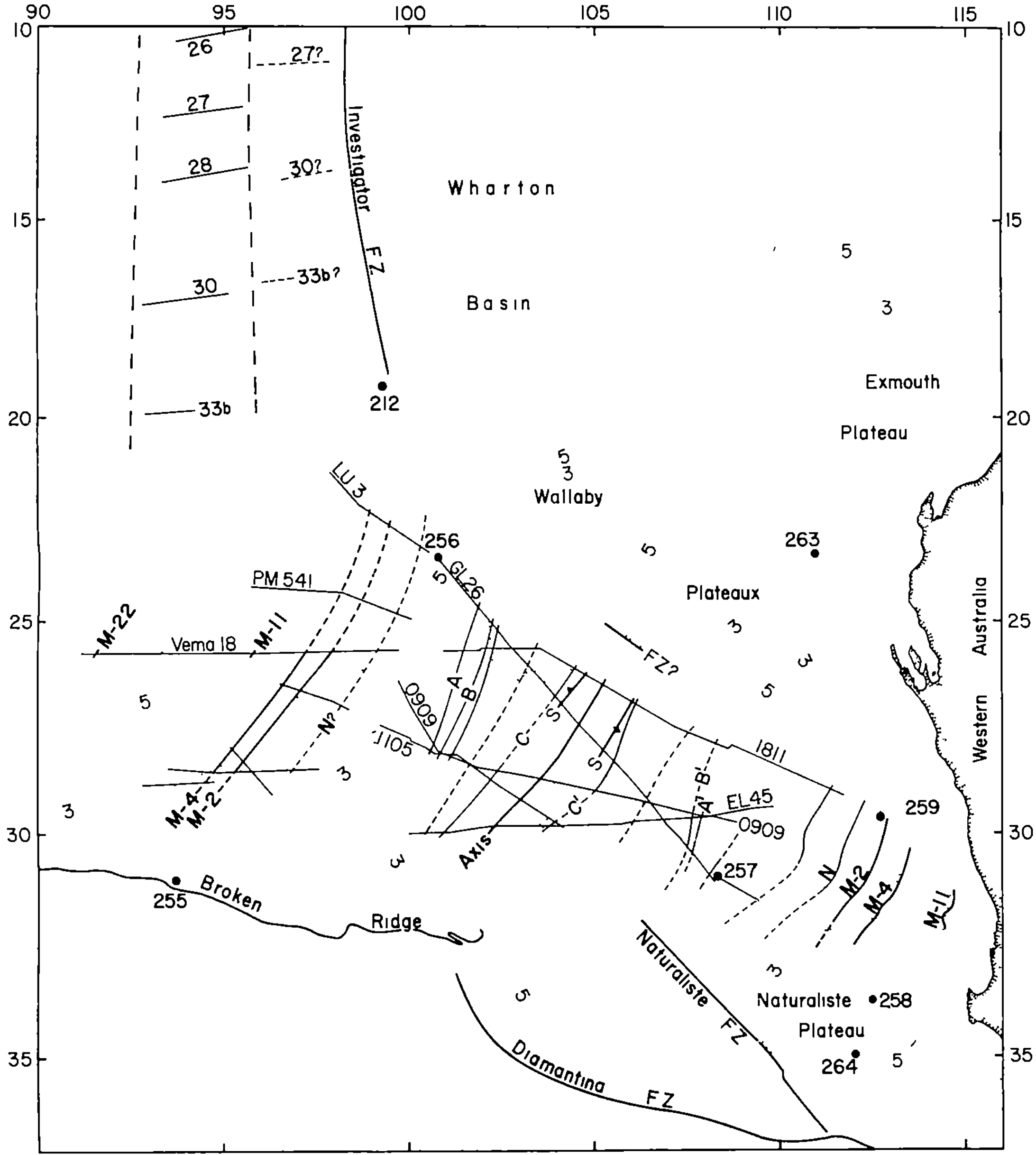

Fig 3 Map of the Wharton basin showing the southern half of the anomaly pattern of Sclater and Fisher[11] (anomalies 26–33b), the Perth and Broken Ridge sequences of Mesozoic (M) magnetic anomalies, and the axis of the proposed spreading centre Magnetic lineations A, A', and so on, are derived from observed profiles shown in Fig 5 Wall Plat, Wallaby Plateau, N, correlated negative magnetic anomaly, dotted lines labelled '3' and '5', the 3,000 and 5,000 corrected meter isobaths respectively, S, basement scarps, unlabelled lines crossing the Broken Ridge lineations, segments of L-DGO tracks on which anomalies M-2 and M-4 were observed, FZ, fracture zones, ●, DSDP site numbers

orientation just before the end of the quiet period, and swept pre-existing 'Tethyan' and Mesozoic crust, and finally its own northern flank, northwards into the Java trench (aided since about 55 Myr BP by the northward spreading of the present South-east Indian Ridge)

The orthogonal relationship between the Perth magnetic lineations and the Naturaliste fracture zone suggests that the Australian continental margin from Perth to the Wallaby plateaux was an initial line of weakness along which transform faulting occurred This geometry also suggests that the northern margin of the Naturaliste Plateau (Fig 1) owes its shape to initial rifting and transform faulting If the identification of the Broken Ridge sequence as Mesozoic-age anomalies is correct, then the oldest crust in the southern Wharton basin (early to middle Jurassic) should lie between latitudes 18°–23°S just east of the Ninetyeast Ridge

Whatever its mode of origin, the existence of the Perth sequence has important ramifications regarding the breakup of eastern Gondwanaland and the evolution of the eastern Indian Ocean The Perth lineations are consistent with the DSDP data and stratigraphical evidence from the coastal basins of Western Australia[7] in that they disallow a gap in Gondwanaland west of Australia, at least in late Jurassic times The seafloor of the region of the inferred gap, that is, the present southern Wharton Basin, is not of Tethyan vintage, but has been created since the breakup of Gondwanaland The Perth sequence by itself provides no indication as to which continental block lay adjacent to Western Australia, but it does prove that the block began to drift away to the north-west before the formation of anomaly M-11 (127 Myr BP) The trend of the Broken Ridge and quiet zone lineations indicates that the continental block

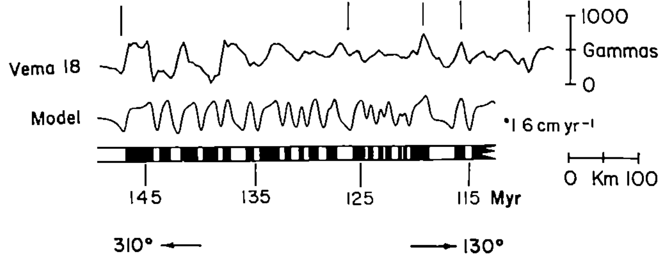

Fig 4 Observed magnetic profile Vema 18 projected perpendicular to the strike of anomaly M-2 and plotted above the model anomaly profile and block model, the corresponding track segment is shown in Fig 3

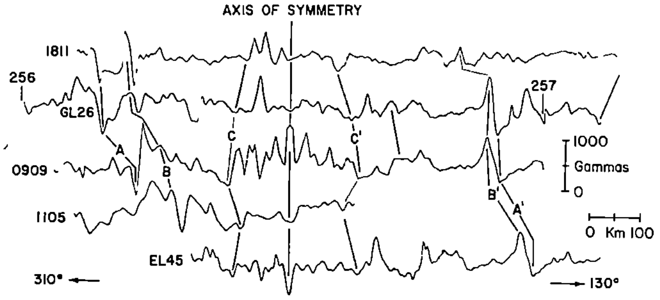

Fig. 5 Observed magnetic anomaly profiles across the proposed spreading centre in the quiet zone between the Perth and Broken Ridge sequences The profiles are projected perpendicular to the axis of symmetry, the corresponding track segments are shown in Fig 3 Anomalies A–A′ to C–C′ correlate, and may be isochronous

in question continued to move in a north-westerly direction The late Jurassic–late Cretaceous spreading centre postulated here indicates that the block could have reached the vicinity of longitude 90°E by the time north–south spreading began adjacent to the present Ninetyeast Ridge[11,20] This scenario strongly suggests that India was the continental block that abutted Western Australia

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Gene duplication in experimental enzyme evolution

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A system to study enzyme evolution experimentally has been developed Multiple mutations are required to improve enzyme specificity and the authors propose that such mutations will be acceptable only in 'silent' gene copies—shown here to be a frequent response to selective pressure

DIVERGENT evolution of an enzyme family from a common ancestor must involve gene duplication as the first step, followed by mutation of one copy to a 'silent gene' that produces an inactive product that cannot fold correctly[1] Natural selection will conserve the sequence of the viable copy but the progeny of the silent gene will become heterogeneous in the population by both mutation and recombination A reversion of the original lesion or a compensating mutation may restore at any time the capacity of the protein to fold correctly and mutations accumulated in the 'silent' phase will then be expressed Natural selection may refine the structure thereafter either back to the old specificity—whereby isoenzymes would result —or to a new specificity, establishing an enzyme family

Some evidence for this hypothesis can be gleaned from the tertiary structures of the serine proteinases, trypsin, chymotrypsin and elastase[2] The conformations of their polypeptide chains are almost superimposable and most of the internal residues are identical or chemically similar Only one or two changes in the surface are adequate to explain their different specificities, but over 80% of the rest of the surface residues are different Even in the highly conserved interiors there are clusters of different residues that seem to provide radically different solutions to the same space-filling problem It is hard to see how these alternative arrangements could have evolved without going through an intermediate that could not fold correctly

The fossil evidence that would support these evolutionary hypotheses has long since been degraded, however, and we are aware of the weakness of arguments based only on comparisons of contemporary structures—even of tertiary structures Some discipline would be imposed on our imagination if we could observe in detail the evolution of an enzyme towards a new specificity under precisely defined selective pressure

Continuous culture of microorganisms in a chemostat represents a closed ecological niche in which the biological fitness of the organism can be measured by the steady-state biomass and concentration of growth-limiting nutri-

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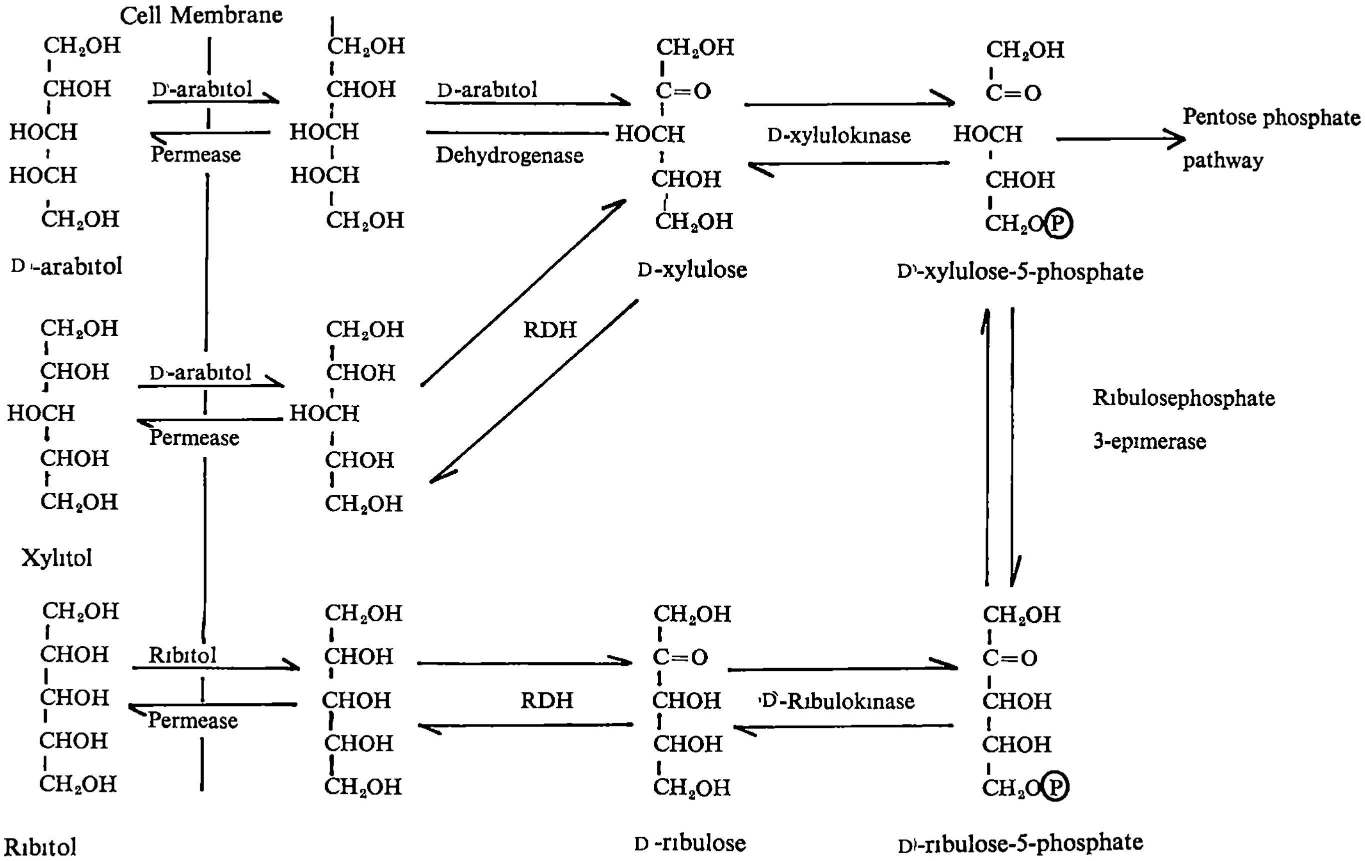

Fig 1 Pentitol metabolism in *Klebsiella aerogenes*

ment in the effluent If the growth-limiting carbon source is a poor substrate for an essential catabolic enzyme, growth may be limited by the activity of this enzyme Mutants with increased enzyme activity will outgrow the ancestral strain and displace them from the niche[3] Such an 'evolutionary step' will be signalled by a rise in steady-state biomass and a fall in effluent substrate level Samples of the 'ancestral' and 'evolvant' cultures can be stored as 'fossils' and batch cultures of these can be grown separately to examine the properties of the enzyme in each strain

Evolution of ribitol dehydrogenase

The pioneering studies of Mortlock and Wood[4] and of Lin[5] and their colleagues on the growth of *Klebsiella aerogenes* on pentitols, established the metabolic pathways shown in Fig 1 The wild type organism will grow on ribitol or D-arabitol as sole carbon source, having an inducible pentitol permease, ribitol dehydrogenase (RDH) (ribitol NAD oxidoreductase E C 1 1 1 c) and D-arabitol dehydrogenase Xylitol and L-arabitol, which are not normally found in nature, will not support growth of the wild type organism, but mutants that are constitutive for ribitol dehydrogenase will grow slowly on these 'unnatural' pentitols Their metabolism has been conclusively demonstrated[6] to be by a side specificity of ribitol dehydrogenase, but whereas the apparent K_m for ribitol was about 1 mM, that for xylitol and L-arabitol was around 1 M It was likely, therefore, that the activity of this enzyme would limit the growth of *K aerogenes* on xylitol in continuous culture, since the pentitol permease seems to transport xylitol efficiently

We therefore began an extensive study of the activity of ribitol dehydrogenase in continuous cultures of a constitutive strain, A, of *K aerogenes* (strain X1 (ref 5)) This was grown in a minimal salts medium with xylitol (0 2% w/v) as sole carbon source in a continuous culture apparatus of our own design and manufacture based on the Porton type fermenter[7] The appearance of better adapted strains was followed by monitoring the bacterial dry weight and residual substrate level in the chemostat

To demonstrate that our system would select putative evolvants we grew an arginine-requiring derivative of strain A on medium supplemented with arginine and guanine, at steady state the chemostat contained approximately 10^{12} cells We then inoculated with 20 cells of a guanine-requiring derivative of strain B (strain X2 (ref 5)) which has a mutant ribitol dehydrogenase with an apparent K_m for xylitol of 0 5 M Twenty cells were used to avoid the statistical chance that the fitter strain would be eliminated[3] Takeover was monitored by measuring normal chemostat parameters (Fig 2*a*) and by plating on appropriate media (Fig 2*b*), these data show that takeover was complete within 7 d

Spontaneous evolution

Our initial experiments (summarised in Fig 3 and Table 1) were performed without mutagenesis to mimic natural evolution Strains A1 and A11 were investigated in detail (Table 1) The apparent maximum specific growth rates on 0 2% xylitol, measured by the washout technique of Tempest[8], confirmed that both strains had increased fitness for their environment The ribitol dehydrogenase activity of strains A, A1 and A11 was measured in cell free extracts obtained by ultrasonication of saturated batch cultures grown on xylitol Neither the apparent K_m for xylitol nor the ratio of xylitol activity to ribitol activity was altered, but a significant increase in total enzyme activity occurred at each step

Is this due to superproduction of wild type enzyme or to evolution of enzymes with increased catalytic efficiency? We purified each of these enzymes to homogeneity by the methods of Taylor, Rigby and Hartley[9], and examined their kinetic parameters There was no detectable change in enzyme activity (Table 1), but it is clear from the purification schedules (Table 2) that strain A1 makes five times

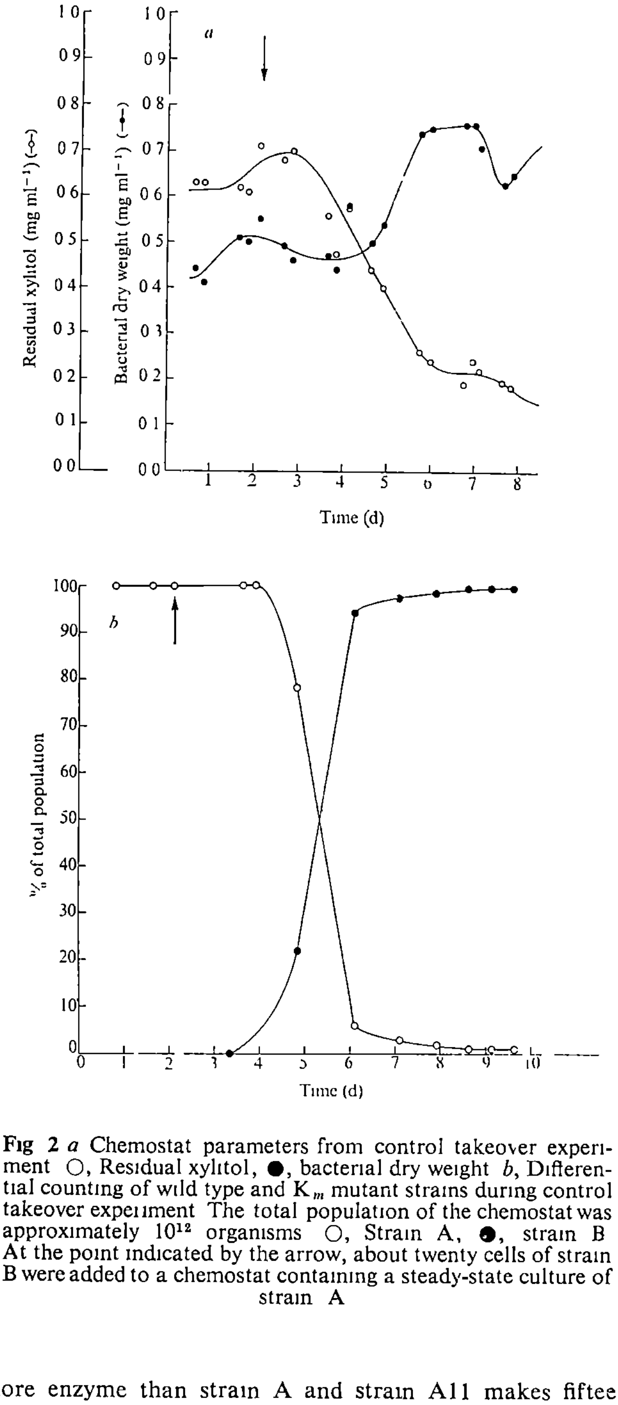

Fig 2 *a* Chemostat parameters from control takeover experiment O, Residual xylitol, ●, bacterial dry weight *b*, Differential counting of wild type and K_m mutant strains during control takeover experiment The total population of the chemostat was approximately 10^{12} organisms O, Strain A, ●, strain B At the point indicated by the arrow, about twenty cells of strain B were added to a chemostat containing a steady-state culture of strain A

more enzyme than strain A and strain A11 makes fifteen times more In the latter case the enzyme represents 17% of the total protein in the extract

Spontaneous evolution gives only increased enzyme production Will specific mutagenesis change this pattern? We built a small ultraviolet lamp into our chemostat and irradiated cultures at a low level such that about 10% of such treatments failed to initiate takeover events The genealogy of fitter strains selected in this way is shown in Fig 3, and Table 1 lists the properties of a typical pair Strain A2 appeared by all criteria to be wild type, but strains A21 and A211 contained elevated levels of enzyme The enzyme was purified to homogeneity from these strains and was kinetically indistinguishable from wild type (Table 1) Similar results were found for all the ultraviolet-induced evolvants shown in Fig 3, 20% of the total protein of strain A6111 was ribitol dehydrogenase Superproduction of wild-type enzyme seems to be the invariable response of the organism to this selective pressure, whether mutations arise spontaneously or are induced by low levels of mutagenesis ●

Enzyme superproduction

Our original hypothesis about evolution of new enzymes demands gene-doubling as the first step Is gene-doubling the explanation for enzyme superproduction, or are other phenomena responsible, such as promoter mutations which lead to high transcription rates for the gene? To distinguish gene-doubled strains we measured the frequency at which mutants lacking ribitol dehydrogenase occurred in strains A, A1 and A11 If the probability of a bacterium carrying a single RDH gene mutating to be RDH^- is P, then that for a bacterium carrying two copies is P^2, for one carrying three copies P^3 and so on This makes the reasonable assumption that each copy will be equally mutable at sites essential for enzyme function

Parallel cultures of each strain were subjected to mutagenesis with N-methyl-N′-nitro-N-nitrosoguanidine followed by a penicillin selection against xylitol Mutants unable to grow on xylitol but able to grow on D-xylose were isolated by replica plating, those which retained the ability to grow on ribitol, being revertants of the constitutive mutation, were discarded This screening procedure produces a set of mutants which contains both RDH and permease negatives, these were distinguished by testing each xylitol-negative clone for ribitol dehydrogenase activity The mutation frequency for the wild type is sufficient to guarantee against the isolation of dependent mutants

Table 3 shows that strains A and A1 have approximately equal mutation frequencies whereas that for strain A11 must be less than the square of the wild type frequency This suggests that strain A11 carries multiple copies of the RDH gene, whereas strain A1 does not

This conclusion was confirmed by segregation analysis Strains which carry two contiguous copies of a gene segregate clones at a high rate with only one copy[10-13] We examined the frequency at which superproducing strains, which give large colonies on 0 05% xylitol plates, produced small colonies after overnight growth on broth A number of these small colonies was picked and assayed for ribitol dehydrogenase activity after growth on casamino acids and ultrasonication Table 4 shows that about one-fifth of the small clones selected from strain A11 had wild type levels of enzyme, giving a segregation frequency of $1\ 4\times10^{-3}$, whereas strain A1 produced no segregants Reversion of a point mutation would occur at a frequency of about 10^{-6}, so strain A11 carries multiple copies of the RDH gene

Strain A211 appears to carry more copies of the gene than strain A11, since it segregates at a higher frequency (Table 4) Some of the A211 segregants have enzyme levels like strain A11, while others are clearly wild type in behaviour (another class A11/A in Table 4, could not be

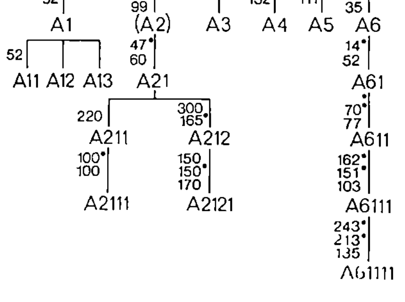

Fig 3 Genealogy of spontaneous and ultraviolet-induced evolvants The strain numbers indicate the evolutionary history of the strain Thus A1 and A2 and so on are derived directly from A, A11 and A12, and so on from A1 The numbers between strains are the generations which elapsed before isolation of the new strain *An ultraviolet mutagenesis

Table 1 Initial experiments to mimic natural evolution performed without mutagenesis

Strain	Apparent μ_m (h^{-1})	Apparent K_m for xylitol in crude extract	Specific activity in crude extract (RHD units mg^{-1} soluble protein)	Apparent K_m for xylitol of pure enzyme	Xylitol ribitol activity ratio for pure enzyme
A	0 53	1 2 M	2 0	1 2 M	0 24
A1	0 64	1 4 M	9 0	1 4 M	0 21
A11	0 82	1 3 M	32 0	1 3 M	0 17
A21	0 64	1 6 M	11 0	—	—
A211	0 84	1 2 M	27 0	1 3 M	0 18

One unit of RDH activity is defined as that amount which reduces 1 μmol NAD min^{-1} in the conditions of the standard assay system, 0 83 mM NAD, 100 mM phosphate buffer, *p*H 7 0, 28° C Apparent K_m values were determined in this system The xylitol ribitol activity ratio is that of the apparent V_{max} on xylitol to the specific activity determined on saturating (50 mM) ribitol

unambiguously assigned as A or A11) These results are those expected if A11 carries two copies of the RDH gene while A211 carries three copies If A211 carried four copies, segregants with an enzyme level between A11 and A211 should have been detected

Comparison of these segregation frequencies with results for duplications of structural genes in *Escherichia coli* suggests that strain A11 may have a duplication covering several genes Segregation frequencies have been measured for *E coli* strains with gene duplications of glycyl-tRNA synthetase[10], glycyl-tRNA[11], tryosyl-tRNA[12 14], and the *lac* operon[15] and the rII region of bacteriophage T4D[13] The segregation frequencies we observe are higher than for strains with a tandem gene duplication[11,14,15], but lower than for strains in which 5% of the genome is duplicated[10] This supposition is supported by the observation that during enzyme purification from strain A11, a second protein was observed to be increased considerably in amount relative to strain A, and acrylamide gels of crude extracts of A11 showed that this protein and ribitol dehydrogenase were present in roughly equal amounts

Table 2 Comparison of RDH purification for strains A, Al and All

Purification step	Total units (× 10^{-5})			Specific activity (RDH units mg^{-1} protein)			Purification			Yield		
	A	A1	A11	A	A1	A11	A	A1	A11	A	A1	A11
Crude extract	1 8	8 8	27 5	1 0	5 1	18 7	1	1	1	100	100	100
Streptomycin supernatant	1 6	6 0	17							92	69	62
35→55% $(NH_4)_2SO_4$ cut	1 0	4 5	11	2 3	18 8	36 5	2 2	3 6	1 9	59	52	39
60 mM DEAE batch eluate	0 75	4 0	8	10 3	74	119	10	14 4	6 4	44	46	29
DEAE gradient eluate	0 7	2 4	4 5	55	87	107	52	16 9	5 8	38	27	16
G-200 Sephadex eluate	0 45			83			80			26		

The figures for total enzyme units are normalised to a preparation from 1 kg of cells

Why make more enzyme?

We have shown that enzyme superproduction is an invariable response to the selective pressure used under conditions of mild mutagenesis, whether spontaneous or induced by ultraviolet light or nitrosoguanidine (J M Dothie and B S H, unpublished) Figure 2 clearly demonstrates that a mutant with increased specificity for xylitol such as strain B (obtained after powerful nitrosoguanidine mutagenesis[6]) would have taken over from strain A in our chemostats Moreover, the growth rate of strain B on xylitol is the same as that of strains A1 and A21, so it would compete effectively with a first-stage superproduction mutant This phenomenon is not an artefact of chemostat selection since we have also found nothing but superproducers by plate selection on low concentrations of xylitol after mild mutagenesis One must conclude that the frequency of mutations leading to increased enzyme production is much greater than that leading to improved enzyme specificity

The rate of spontaneous mutation to RDH^- for this organism is about 10^{-6} There are 249 residues per chain, thus 250 triplets in the gene and each triplet can yield on average six amino acid replacements by a single base change Therefore the frequency of any particular single-step replacement will be of the order of 10^{-9}, assuming equal mutability across the gene Values of this order have been observed for *E coli* genes, ranging from 2×10^{-10} for streptomycin resistance[16] to 10^{-8} for bacteriophage T1 resistance[17]

We have screened some 10^{14} wild type organisms without detecting mutational alteration of the enzyme's specificity for xylitol If such mutational improvement could result from a single base change it would occur at a frequency greater than 10^{-10} Statistical considerations allow that some mutations will not be detected in the chemostat[3], but we calculate that we should detect around 10% and certainly more than 1% of all events We conclude that no point mutation can lead to a viable enzyme with improved specificity for xylitol

We are forced to conclude that this ribitol dehydrogenase cannot evolve towards a new specificity for xylitol by single step mutations whilst under continuous selective pressure Multiple mutations may, and do (J M Dothie and B S H,

Table 3 Determination of mutation frequencies

Strain	No of xylitol negative clones	No of RDH negative clones	Frequency of RDH negative clones
A	71	43	2 6 × 10^{-4}
A1	30	18	6 0 × 10^{-4}
A11	0	0	<3 0 × 10^{-8}

Each strain was grown on B broth to a density of 2 × 10^8 cells ml^{-1} then washed with and resuspended in Tris-maleate, *p*H 6 0, nitrosoguanidine was added to a final concentration of 0 2 mg ml^{-1} and the culture incubated at 37° C for 30 min The cells were then washed with B broth, diluted 1 20 into the same medium and grown out overnight After being washed with M9 and then starved for 20 min the culture was diluted with M9 to give a density of 10^7 cells ml^{-1} Xylitol was added to 0 2% and benzylpenicillin to 0 6 mg ml^{-1} and the culture was incubated for 4 h Appropriate dilutions were spread on to M9/xylose plates which after incubation were replicated on to M9/xylitol plates

Table 4 Results of segregation experiments

Strain	Small clones (%)	%	Segregants No	Class	
A1	0 03	0	0	—	
A11	0 76	0 14	28	A11	(23)
				A	(5)
A211	0 75	0 68	32	A211	(3)
				A11	(13)
				A11/A	(9)
				A	(7)

After being cloned several times on xylitol, strains were grown on B broth until the optical density at 650 nm was approximately 1 0 Aliquots of a suitable dilution were spread on to M9/0 05% xylitol plates which after incubation were examined and the number of small colonies determined A sample of these small colonies was picked and assayed for RDH level Cultures were grown on 3% casamino acids and the cells were ruptured by treatment with lysozyme/EDTA and Triton X-100 This procedure is as effective at releasing RDH as ultrasonication Extracts were then assayed for RDH activity and total protein and from the specific activities thus determined each clone could be classified

unpublished), produce enzymes with improved specificity, but these would occur spontaneously at a very low frequency—around 10^{-18} Spontaneous steps which increase enzyme level, however, are more frequent—around 10^{-13}—and most of these steps are due to gene multiplication Thus far, therefore, these experimental results are in accord with the evolutionary hypothesis propounded above Further experiments are needed to test the evolutionary pathway of the gene-doubled strains under conditions of fluctuating selective pressure

If it is generally true that multiple mutations are required for evolutionary improvement, then it is likely that such mutations will often be incompatible with the tertiary structure of the protein Evolutionary progress and viability can be reconciled by gene duplication, which allows the second gene copy to accumulate mutations while not immediately constrained by natural selection We have demonstrated that multiple mutations are required for, and gene duplications are a frequent part of, one evolutionary pathway

Opposite conclusions can be drawn, however, from the studies[18] of Clarke and her collaborators on the experimental evolution of an amidase in *Pseudomonas aeruginosa* Whereas the wild type enzyme is specific for acetamide and propionamide, new specificities for butyramide, valeramide, phenylacetamide and acetanilide evolve readily by single mutations We suspect that this apparent contradiction is explained by the fact that we are trying to set a more difficult evolutionary problem for a more complex enzyme a change in stereospecificity about the carbon atom adjacent to the site which donates hydride ion to NAD But the contrast between the conclusions which we draw strongly indicates that the evolutionary potential of an enzyme may itself be a function of its tertiary structure

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letters to nature

Black holes of small mass

ANY star which has exhausted all of its nuclear fuel and which cannot eject sufficient matter so that its mass becomes less than roughly two solar masses, must undergo gravitational collapse to become a black hole Thus one usually associates black holes with masses of stellar proportions But it is possible that during the initial chaotic conditions of the Universe, black holes of much smaller mass[1,2], down to 10^{-5} g, may have been formed Here we suggest an experiment by which one may detect or set meaningful upper limits to the number density of black holes of small mass (hereinafter referred to as BHSM)

First we estimate the BHSM–Earth collision time, τ, under reasonable assumptions, for various values of the mass *m* of the BHSM Assume the space density of BHSM is ρ g cm^{-3} Because the mass distribution of the BHSM is unknown, we may assume that they all have the same mass *m*, and investigate the consequences of varying the mass Then[3],

$$\tau^{-1} = \pi R_E{}^2 \rho V \left[1 + (V_e/V)^2\right]/m \qquad (1)$$

where R_E is the radius of the Earth, V_e ($\approx 10^6$ cm s^{-1}) is the escape velocity from the Earth, and V the velocity of the BHSM at infinity Because of the solar motion[1] and the Earth s rotation, $V \gtrsim 10^6$ cm s^{-1} So $(V_e/V)^2 \lesssim 1$ and

$$\tau \approx m/\pi R_E{}^2 \rho V \approx 10^{-10}\, m\, (10^7/V)\, (10^{-23}/\rho)\ \text{yr} \qquad (2)$$

The parameter *m* may be varied in order to estimate the maximum possible flux of BHSM and to ascertain whether such a flux is detectable

Highly sensitive superconducting accelerometers[5,6] are now being developed for use in low temperature gravitational wave detectors One of us (D G B) is constructing an accelerometer which is also particularly applicable to the detection of BHSM This accelerometer measures the deflection of a magnetically levitated test object of mass M against a constraining magnetic field Deflections Δx as small as 10^{-17} cm may be detected To detect a BHSM we must observe a differential acceleration of the test mass relative to its support Suppose the accelerometer is supported by a rigid body so that the test mass is at a distance l from the centre of mass C Consider the passage of a BHSM of mass m with speed V_0 at a distance s from the test mass and $(s+l)$ from C Then, neglecting geometrical factors, the test mass gains an impulsive velocity[7] $2Gml/V_0s(l+s)$ relative to C Because the test mass is attached to the accelerometer by a magnetic 'spring' of 'spring constant' $M\omega^2$

$$\tfrac{1}{2} M\omega^2 (\Delta x)^2 = \tfrac{1}{2} M\,[2Glm/s(s+l)V_0]^2$$

so that

$$m = V_0\,\omega\,(\Delta x)s(s+l)/2Gl \tag{3}$$

We note that the mass on which the accelerometer is mounted need not be a gravity wave antenna, and that ω is not related to the frequency of the antenna The strength of the magnetic field may be varied to vary the spring constant $M\omega^2$ The value of ω can vary from 10^{-2} rad s^{-1} to 10^4 rad s^{-1} For greatest possible sensitivity, we take $\omega = 10^{-2}$ rad s^{-1} and $\Delta x = 10^{-17}$ m

For BHSM to be observable, we require a flux sufficient to produce one event per year Setting $\tau R_E^2/ns^2 = 1$ yr in equation (2) we require that

$$m \lesssim 2.4 \times 10^{-8}\, n\, s^2 (\rho/10^{-23})\,(V/10^7) \tag{4}$$

where n is the number of detectors

For simplicity we separate two cases, $s \leqslant l$ and $s > l$ If $s \leqslant l$, equation (3) reduces to $m = \omega(\Delta x)sV_0/2G$ to within a factor of two Now if $V > V_e$ it follows that $V_0 \sim V$ So to detect a BHSM travelling within a distance s it is necessary that

$$m \gtrsim 7.5 \times 10^{-6}\, s\,(V/10^7)\ \mathrm{g} \tag{5}$$

Inequalities (4) and (5) can both be satisfied only if

$$s \gtrsim (3.1 \times 10^2/n)\,(10^{-23}/\rho)\ \mathrm{cm}$$

If the apparatus is to be isolated, l is restricted to about 10^3 cm Taking $s \leqslant 10^3$ cm and choosing $n = 100$ it follows that

$$\begin{aligned} 2 \times 10^{-5}\,(10^{-23}/\rho)\,(V/10^7) \lesssim m \lesssim\ & \\ 2(\rho/10^{-23})\,(V/10^7)\ & \end{aligned} \tag{6}$$

So the range of masses that can be detected depends on ρ and V We note also that equation (6) can be satisfied only if $\rho \gtrsim 10^{-25}$ g cm^{-3} It is likely that during the early history of the Universe BHSM became concentrated into galaxies with a space density ρ_G An upper limit[8] for ρ_G in the solar neighbourhood is about 10^{-23} g cm^{-3} If ρ_G equals this upper limit and $V \sim 10^7$ cm s^{-1}, masses in the range 10^{-5} to 1 g may be detected

If the Earth itself is the supporting mass $l = R_E$ Taking an upper limit for s of 10^8 cm one requires that $\rho \gtrsim 10^{-30}$ g cm^{-3} If ρ_c be the cosmological space density of BHSM then[2], $\rho_c \lesssim 10^{-28}$ g cm^{-3} If this upper limit is attained and $V \sim 10^7$ cm s^{-1}, masses in the range 1 to 10^5 g may be detected In this case narrow band filtering of the seismic noise at the frequency corresponding to the interaction time of the BHSM would be necessary in order to reduce the noise level Such filtering could be difficult

For the case $s > l$, from equation (3) we have that $m \approx \omega(\Delta x)V_0s^2/2Gl$ to within a factor of two, so that masses in the range

$$\begin{aligned} 7.5 \times 10^{-6}\,(V/10^7)\,s^2/l \lesssim m & \\ 2.4 \times 10^{-8}\, ns^2\,(\rho/10^{-23})\,(V/10^7) & \end{aligned} \tag{7}$$

may be detected provided $\rho/10^{-23} \gtrsim 3.1 \times 10^2/nl$ For example if $n = 100$, $l = 10^3$, $V = 10^7$ cm 5^{-1} and $s > 10^3$ cm, BHSM can be detected if $\rho \gtrsim 10^{-26}$ g cm^{-3}

We now consider whether BHSM can be concentrated within the Solar System through gravitational interaction with a planet such as Jupiter This could give rise to an even larger flux of BHSM The collision time τ_J of a BHSM with Jupiter is obtained by multiplying equation (2) by $(R_E/R_J)^2$ where R_J ($\approx$ 0.3 AU) is the radius of the sphere of influence[9] of Jupiter So

$$\tau_J = 2 \times 10^{-18}\, m\,(10^7/V)\,(10^{-23}/\rho_G)\ \mathrm{yr} \tag{8}$$

Thus frequent collisions are possible for reasonable parameters in equation (9) Suppose that a (net) fraction α (V) of BHSM coming within the sphere of influence of Jupiter are captured by the Solar System The contribution of these BHSM to the solar system density is

$$\begin{aligned} \rho_S &= \alpha\,(5 \times 10^9/\tau_J)\ [3\,m/4\pi\,(7.5 \times 10^{13})^3] \\ &\approx \alpha\,(V/10^7)\,(\rho_G/\,10^{-23}) \times 10^{-15}\ \mathrm{g\,cm^{-3}} \end{aligned}$$

The value of $\alpha(V)$ is difficult to estimate, but, if $\alpha \gtrsim 10^{-8}$ some increase in ρ_S over ρ_G can be obtained But $\rho_s \lesssim 10^{-18}$ g cm^{-3}, since otherwise perturbations of spacecraft would have been discovered It has been suggested[10-12] that the Tunguska meteoric event was caused by a black hole of mass $\sim 10^{21}$ g We note that even if $\rho_s = 10^{-18}$ g cm^{-3}, such an event was an extremely rare occurrence (equation (2))

The trajectory of a BHSM can be roughly estimated by utilising the uniaxial directional properties of the accelerometer[5,6] Two detectors properly oriented could distinguish between particles moving up or down Since BHSM can travel through the Earth whereas meteors cannot, those particles travelling upwards must be black holes

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Particle acceleration in planetary magnetospheres

MEASUREMENTS of energetic particle fluxes in space[1,2] have stimulated interest in the problem of particle acceleration inside planetary magnetospheres Suggestions that terrestrial auroral (keV) electron precipitation is associated with transient space charge effects[3], have been discarded, largely because the parallel (plasma) electrical conductivity ($\sigma_{\parallel}$) is so large that magnetospheric field lines are constrained to remain equipotentials[4]

Here I point out that planetary magnetospheric field lines are not constrained to remain equipotentials when the plasma conductivity ($\sigma_{\parallel}$) is dominated by current driven microinstabilities as opposed to electron-ion collisions For example in the 'collision-free' regime defined by $c_s \leqslant u_{\parallel}^- \leqslant v_{Te}$ and $T_e \gg T_i$, the plasma conductivity $\sigma_{\parallel}$ is determined by the spectrum of unstable ion-sound waves The measured value is [5]

$$\sigma_{\parallel} \simeq \omega_{p_e}(4\pi \times 10^8)\ \text{mho m}^{-1} \qquad (1)$$

$c_s \equiv \sqrt{(kT_e/M_i)}$=ion-sound speed, $v_{Te} \equiv \sqrt{(2kT_e/m_e)}$=electron thermal velocity, $u_{\parallel}^-$=relative drift velocity between the electron and ion populations, $\omega_{p_e} \equiv \sqrt{(n_e e^2/\varepsilon_0 m_e)}$=electron plasma frequency

If $n_e \sim 10^8$ m^{-3}, $T_e \sim 10$ eV and $u_{\parallel}^- \sim v_{Te}/20$ (where $u_{\parallel}^-$ is induced by transient space charges) then the associated electric field $E_{\parallel}^{(0)} = en_e u_{\parallel}^-/\sigma_{\parallel} \sim 4 \times 10^{-3}$ Vm^{-1} For a flux tube or magnetic field line of length $l \sim 5 \times 10^8$ m (such as a Jovian dipole field line at $L \simeq 3$) the parallel potential $\phi_{\parallel}^{(0)}$ between the equatorial and ionospheric regions is very high, $\phi_{\parallel}^{(0)} \simeq E_{\parallel}^{(0)} l \sim 10^6$ V

The low parallel electron mobility (equation (1)) is the result of resonances between the current carrying electrons and the unstable, current driven, ion-acoustic modes[6], in fact an 'effective' collision frequency (Ω), due to the turbulent electrostatic ion-sound fluctuations, can be defined where[6]

$$\Omega = (2\pi e^2/u_{\parallel}^- \varepsilon_0 n_e m_e^2)\Sigma(\mathbf{k}/k^2) W_k \int d^3\mathbf{v}\mathbf{k}\,(\partial f_i/\partial \mathbf{v})\,\delta(\omega - \mathbf{k}.\mathbf{v}) \qquad (2)$$

W_k=electrostatic energy per unit volume in the mode $\mathbf{k}$, $\delta(\omega - \mathbf{k}\,\mathbf{v})$ indicates wave-particle resonance, the rest of the notation is standard Because ion-sound turbulence is not isotropic, however, a small fraction δf_e of the electron population is not in resonance with the unstable modes and consequently it is not included in the summation (2) The number density of this component for a 'shifted' Maxwellian

$$f_e = n_e(\beta/\pi)^{3/2} \exp - \beta[v_{\perp}^2 + (v_{\parallel} - u_{\parallel}^-)^2]$$

(with $\beta \equiv m_e/2kT_e$) is

$$\delta n_e = \int d^3 v \delta f_e$$

$$= n_e(\beta/\pi)^{3/2} \int_0^{2\pi} d\theta \int_{-\infty}^{+\infty} dv_{\parallel} \int_0^{v_{\parallel}\alpha} dv_{\perp} \exp - \beta\left[v_{\perp}^2 + (v_{\parallel} - u_{\parallel}^-)^2\right]$$

$$\simeq (n_e/2)(c_s/u_{\parallel}^-)^2 \qquad (3)$$

(with $\alpha \equiv \sin^{-1}(c_s/u_{\parallel}^-)$)

Assuming that the turbulent electric field $\mathbf{E}^{(1)}$ experienced by the nonresonant electron component δf_e is stochastic, their motion can be described by a Fokker–Planck equation Further, provided that $\langle \mathbf{E}^{(1)}(r_0(t),t) \rangle = 0$, ($r_0(t)$=unperturbed particle trajectory and $\langle\ \rangle$ indicates an ensemble average) the Fokker–Planck equation can be reduced to a simple diffusion equation

$$\{\partial/\partial t + v_{\parallel}\,\partial/\partial r - (e/m_e)\,E_{\parallel}^{(0)}\,\partial/\partial v_{\parallel}\}\delta f_e = \partial/\partial v_{\parallel}\,(D\partial/\partial v_{\parallel}\,(\delta f_e)) \qquad (4)$$

(D=velocity space diffusion coefficient due to the turbulent fluctuations)

It is apparent from equation (4) that the nonresonant component δf_e retains its high parallel mobility and in spite of the turbulence these electrons essentially free fall in the large electric potentials $\phi_{\parallel}^{(0)}$

The combination of high parallel electric potentials $\phi_{\parallel}^{(0)}$ (due to 'anomalously' low conductivity effects) and a tenuous but highly mobile electron component δn_e (due to the nonisotropic nature of ion-sound turbulence) provides an important mechanism for generating sporadic and stormy outbursts of energetic electrons inside planetary magnetospheres

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High power acoustic radar

THE range and sensitivity of turbulence detection by acoustic probing of the lower atmosphere initiated by McAllister *et al*[1] has in part been restricted by the use of low power acoustic pulses (generally ~ 100 W) We shall briefly describe a pulsed high power (8 1 kW input) sound

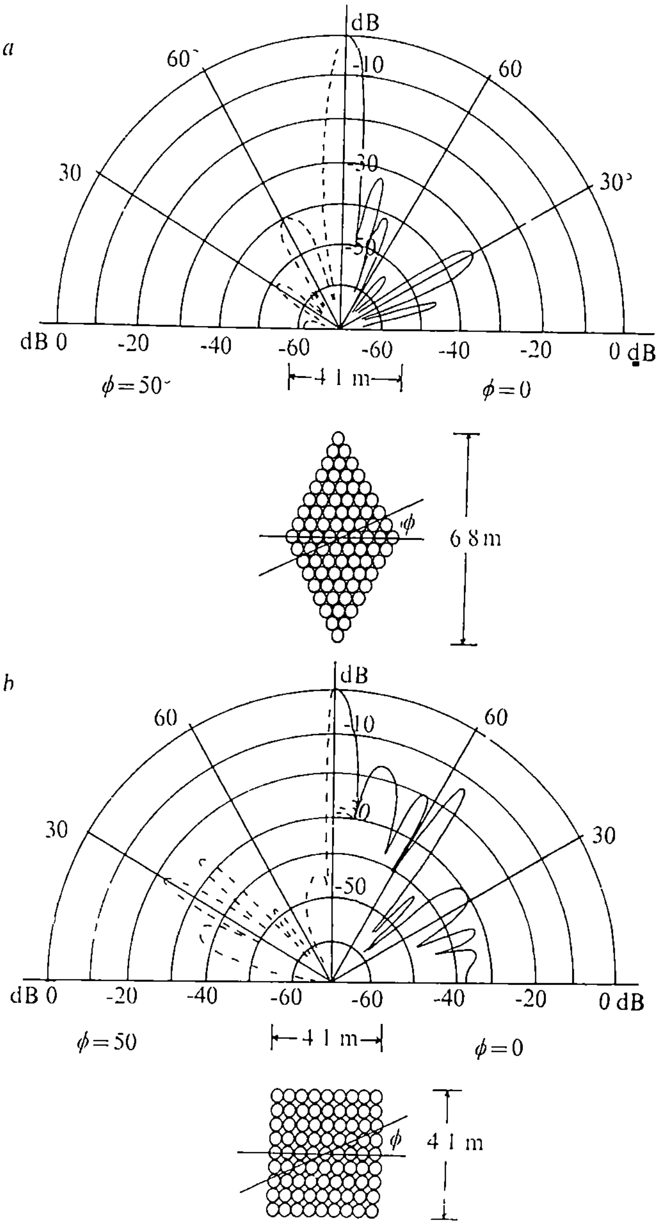

Fig 1 Radiation patterns for, *a* diamond and, *b*, rectangular arrays at 1,305 Hz The solid curve represents half the cross section at φ =0° and the dotted curve is half the cross section at φ =50°

radar system and present representative data obtained with a 2 7 kW system

An all-weather capability coupled with a high degree of system reliability and flexibility was essential The limitations[2] to an acoustic radar and cost estimates led to a suitable operation frequency of approximately 1 kHz

A high gain array was selected as the transmitter antenna An individual transmitter array element consisted of a bin, resonant at a suitable frequency fed by an inverted 100 W electrodynamic transducer exponential horn combination (approximately 50% efficient) These transducers had proven reliabilities and the unit inversion provided the necessary transducer weather protection

Various array configurations were numerically simulated to determine an optimum narrow beamwidth or gain and side-lobe level appropriate for an acceptable amount of noise pollution An 81 element diamond-shaped array was finally selected and its beam pattern, shown in Fig 1, indicates that this design has better side-lobe characteristics than a conventional rectangular array The fact that the beam pattern is not symmetrical is not of any serious consequence Note that only two cross sections are shown and that the patterns presented consist of the calculated space factor (array beam pattern) multiplied by the measured radiation pattern of an individual array element The actual radiation pattern of the array has not yet been measured and effects such as atmospheric turbulence upon the beamshape are unknown To further improve side-lobe levels, the entire transmitting array was placed in a hole 8 feet deep Each array element will finally be driven by a separate 100-W audio amplifier, giving a total power input of 8 1 kW The results presented here were obtained with a power input of 2 7 kW

The receiving system uses a fibreglass parabolic dish, 5 feet in diameter, incorporating a transducer–horn element as the primary feed This provides a broad receiving beamwidth and thus lessens signal loss due to translation of the sound pulsed by the mean wind profile The dish was placed in a separate hole 8 feet deep (located 40 feet from the centre of the transmitter array) to isolate the receiver from wind noise and general background surface interference A low noise preamplifier and narrowband tuned receiving system was used to amplify the acoustic returns and after detection a signal proportional to the logarithm of the detected signal was used to intensity modulate a chart record Provision was made to vary the sensitivity of the receiving system as a function of time after the transmitted pulse, thus reducing the range of intensities that need be recorded

The system was located in flat open country at Laverton, Victoria, which is traversed by the easterly flow of high pressure and cold front systems which pass across southern Australia The results presented provide a typical cross section of records obtained during the period from mid December 1973 to mid February 1974, when the frequency of operation was 1,350 Hz, the bandwidth 5 Hz, and the pulse length 0 4 s

The record shown in Fig 2*a* was obtained after a cold front had passed the site The development of rising echo structures is a characteristic typically associated with a cold front, in this example the highest echo is observed at a range of 9,500 feet at 0000 EST From 0330 EST, at 7,000 feet an echo associated with subsidence inversion conditions is apparent The arrowed bands represent periods during which the noise created by rainfall saturated the receiving system Conditions during the approach of the centre of the following high pressure system are presented in Fig 2*b* The existence of a high degree of turbulence between the ground and the subsidence inversion is clearly indicated even during the night From 0800 EST with the advent of surface heating, plume activity developed which was capped by the inversion at 4,300 feet

A brief assessment of the relative merit of a 100-W input acoustic sounder compared with the high power sounder is shown in Fig 2*c* The system parameters lead to the high power radar having a signal-to-noise ratio (voltage) gain of approximately 10 over the low power sounder, and for the purposes of demonstration, the whole system was switched between power inputs of 100 W and 2 7 kW Every 2 h the system operated in the low power

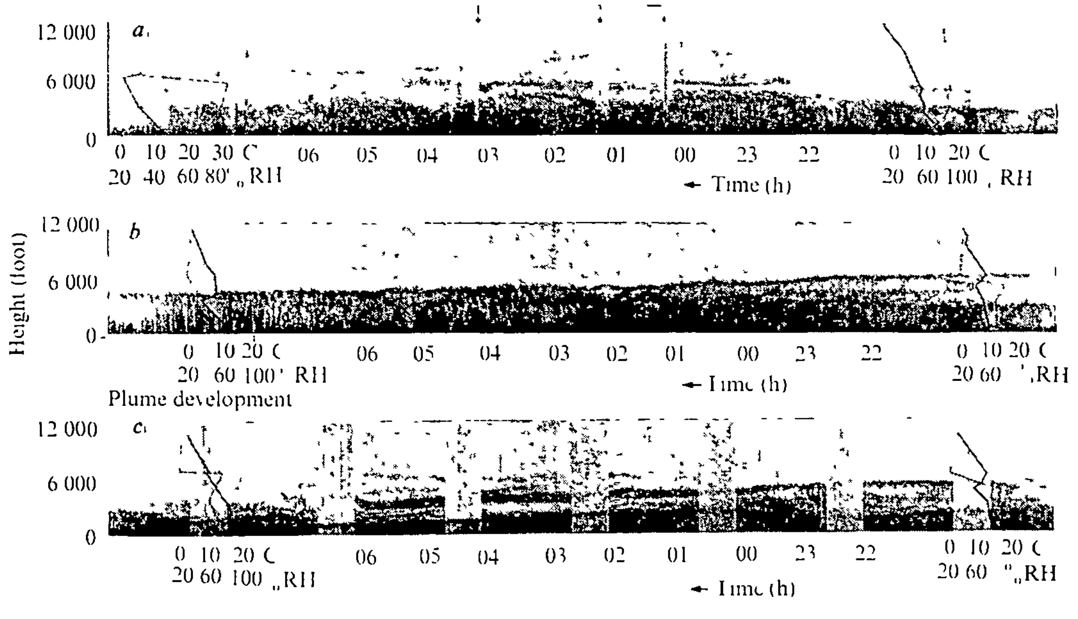

Fig 2 High power acoustic radar record, *a*, obtained after the passage of a cold front on December 16, 1973, *b*, showing night-time turbulent structure during subsidence conditions on December 18, 1973 *c*, Record demonstrating the increased sensitivity of the 2 7-kW input acoustic radar in comparison with the 100-W acoustic radar on February 9, 1974 The abscissa represents time (increasing from right to left), the ordinate represents echo range and the intensity represents the strength of the acoustic return Every half hour range marks at intervals of 500 feet are superimposed upon the record Standard 0815 FST and 2015 FST Weather Bureau radiosonde temperature (solid line) and relative humidity (RH) (dotted line) profiles have also been placed on the records for comparison

mode for a half-hour period (as from 0215 to 0245 EST) This record obtained during high pressure conditions clearly shows a substantial echo regime not indicated by the lower power unit

These preliminary results clearly demonstrate the increased potential of high power acoustic probing The extension to full power of 8 kW will allow the sounder to be used for high resolution acoustic sounding as well as for studying the effects of nonlinear sound propagation in the atmosphere By measuring the change in reflection of electromagnetic energy due to the high power sound slightly altering the dielectric constant of the air, the derivation of temperature profiles is also feasible

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Diurnal change in the location of scintillations in the F-region of the ionosphere

FIELD-aligned irregularities in the F-region of the ionosphere cause amplitude and phase scintillations of satellite radio transmission[1-5] It seems that scintillations increase in magnitude and number towards the northern and southern auroral zones The latitudinal variation is, however, masked by the enhancement of scintillations whenever the angle between the ray path and the magnetic field—the aspect angle—is small Thus, at recording stations from subauroral[4,6] to low[2] latitudes, strong scintillations are observed towards the equator

So far, studies of the amplitude and phase of the scintillations have used VHF beacon transmissions from one or two satellites, and, consequently, consecutive radio scans of the local ionosphere have been relatively infrequent It has therefore been difficult to trace changes in the location of regions of scintillation, at short time intervals Here, I discuss the advantages of using a multisatellite radio transmission system for detecting changes in the location of scintillations at night

The study was based on recordings of the amplitude of radio signals from five United States Navy Navigational Satellites (NNSS) transmitting at a frequency of 150 MHz The satellites move along polar orbits and provide frequent radio scans of the local ionosphere The recording station was situated at Brisbane (27°S, 152 9°E, and 35 8°S geom lat) The receiving antenna had a broad, isotropic beamwidth pattern which made it possible to record satellite passes at high zenith angles of up to 90° As amplitude scintillations at Brisbane are mainly a night time phenomenon[1,2] the recording period was largely limited to a time interval from 1800 to 0500 LT It was possible to record, on average, seven NNSS passes during this time interval Between November 22, 1973 and January 16, 1974, 347 satellite passes were recorded

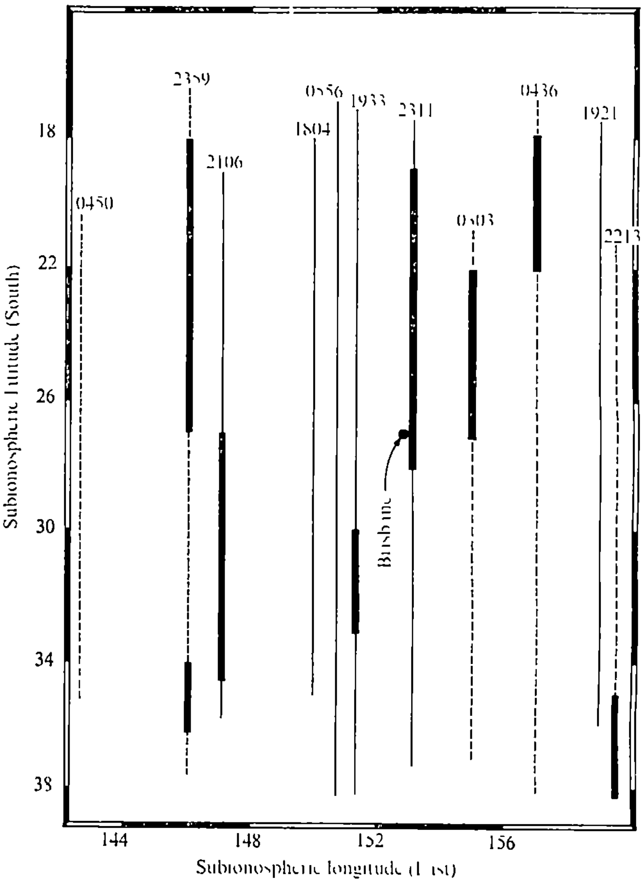

Fig 1 Subionospheric orbits of NNSS satellites Solid line, Night of December 7–8, 1973, broken lines, night of January 8–9, 1974, thick solid lines provide an indication of the horizontal extent of the scintillations The time of the nearest satellite approach (150° EST) is indicated for each pass (LT)

Consecutive radiosatellite scans of the local ionosphere at a height of 400 km were obtained on two nights December 7–8, 1973 and January 8–9, 1974 (Fig 1) It can be seen that the passes recorded at 1804 and 1921 LT on the first night did not reveal any scintillation activity A pass at 1933 LT indicated, however, the presence of a well developed scintillation region polewards of the station Subsequent scintillations at 2106 LT extended more towards the equator Finally, strong scintillations extending well towards the equator were recorded at 2311 LT No scintillations were detected during a morning pass, at 0556 LT A similar development in the location of scintillations was evident during the second night Then, the first recorded pass at 2213 LT indicated scintillations towards the pole The second pass, at 2359 LT, indicated the presence of two scintillation regions towards both the equator and the pole with respect to the station Scintillation activity was limited to regions between the station and the equator during subsequent passes at 0303 and 0436 LT No scintillations were detected during a pass at 0450 LT

The development of scintillation activity tended to follow the pattern shown in Fig 1 Thus, scintillations during the evening occurred between the station and the pole, at high zenith angles Later, but still before midnight, the scintil-

Table 1 Number of nights during which displacements in the location of scintillations were observed

	Time (150 EST) of scintillations	
	1800 – 2400	0000 – 5000
Displacement towards equator	22	4
Displacement towards pole	2	1

lation regions split one remained towards the pole, at somewhat lower zenith angles and the other region shifted towards the equator Finally, scintillation towards the equator became predominant during the morning hours

Table 1 shows the number of nights on which displacement of scintillation regions towards the equator occurred In all but four cases the displacement of scintillations towards the pole, occurred before midnight LT The number of displacements towards the pole is also shown It can be seen that scintillations were first recorded between the station and the equator and were noted subsequently polewards of the original position, the reverse trend was infrequent (Table 1)

The presence of scintillations at high zenith angles and polewards of the recording station is a common phenomenon for Australian stations[2] The spatial effect seems to be strongly dependent on azimuth, and indicates a distinct ridge in scintillation activity This work shows that the polewards ridge structure may drift towards the equator during the evening hours A similar scintillation drift was found in the northern hemisphere by Kersley *et al*[6] who reported that F-region scintillations moved towards the equator between 1500 and 0200 LT

It is likely that the scintillations near the pole are associated with field-aligned irregularities at higher latitudes These irregularities cause pronounced scintillation effects once they have drifted to the position at which the aspect angle is small

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A modified predrift fit of Greenland and western Europe

THE most commonly used predrift fit of north-western Europe against Greenland is based on a least squares fit of the Continental crustal edges which are assumed to follow the 500 fathom submarine contour[1] Recent geophysical studies in the North Atlantic and the Norwegian Sea have established a number of facts bearing directly on the Palaeocene palaeogeography of these areas, and on departures of the continental crustal edge from the 500 fathom contour

The whole of the Rockall–Hatton Plateau is a submerged continental fragment[2] Subsidence of this area commenced in the late Palaeocene (56 Myr BP) and the basement dropped to 600–700 m below sealevel by early Eocene times[3] (51 Myr BP) The topographic expression of the western edge of the plateau is closely coincident with anomaly 24 (60 Myr BP) indicating that rifting between Greenland and Rockall began at this time

The Rockall Trough came into existence before late Senonian times, that is, before anomaly 32 (78 Myr BP), (ref 3) Whether the Rockall Trough owes its origin to seafloor spreading or to subsidence of a continental block remains an unresolved question but it is now certain that it was in existence for at least 18 Myr before any spreading on the Reykjanes Ridge, and probably for a great deal longer

There is a gap of approximately 100 km between the continental slope of Greenland (marked by the 1,000 m contour) and the western limit of the oceanic crust (marked by anomaly 24)[3] in the area south of latitude 55°N This may be interpreted in three ways either the intervening area is one of subsidised continental crust, or there is older oceanic crust between anomaly 24 and the continental slope and therefore an extinct spreading axis which is not in evidence elsewhere in the North Atlantic, or this area was produced by some other, unknown process The first of these hypotheses seems the most conservative

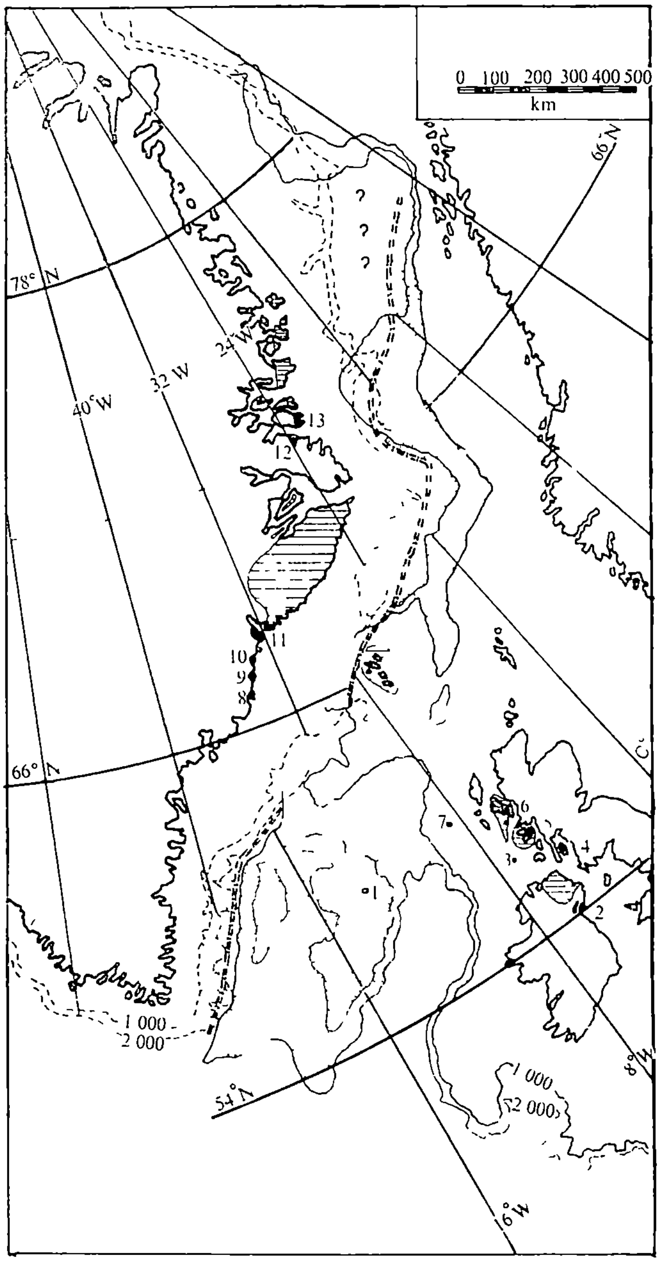

Fig 1 Predrift reconstruction of Greenland and Europe Stippled areas, subsided continental crust, ruled areas, tertiary flood basalts, – – –, 1,000 and 2,000 m bathymetric contours around Greenland, ——, 1,000 and 2,000 m bathymetric contours around Europe, = =, geophysically defined continental margin Tertiary plutonic bodies and ring complexes are numbered 1, Rockall, 2, Mourne 3, Blackstones Bank, 4, Arran, 5, Mull, 6, Skye, 7, St Kilda, 8, Cape Gustav Holm, 9, Nualik, 10, Idglutarajik, 11, Skaergaard, 12, Werner Bjerge, 13, Cape Simpson

in the light of available data from the North Atlantic, and so the continental margin of Greenland is assumed to be coincident with anomaly 24 in this region

The Faeroe Islands are underlain by continental crust[4] The junction of the continental crust and the anomalous crust of the Iceland–Faeroe Rise lies approximately 50 km north-west of the Faeroes and is marked by a large gravity anomaly[5,8]

The magnetic 'Quiet Zone' of the eastern Norwegian Sea is underlain by deeply subsided continental crust[7,8] Between 64°N and 68°N the continental edge is well defined by gravity highs immediately to the seaward side, which are coincident with the limit of magnetic strip anomalies in the area South of this region, however, the edge is less well defined and is now considered to be at the limit of definable strip anomalies

Using available information on the geophysically defined continental edge, I have attempted another fit (Fig 1) This is a subjective reconstruction based on the criterion that areas of known or suspected continental crust should not overlap The only area in which the margins of the continental masses on opposite sides of the Atlantic are well defined is in the region south of Iceland where anomaly 24 is composed of linear features[3] When these predrift isocrons are superimposed the model has only one degree of freedom—relative movement is restricted to a direction parallel to the linear features Further north, the Jan Mayen and Greenland Fracture Zones apply a restraint to this movement, because the fit must bring into juxtaposition those portions of the features closest to the continents Thus, the obtained fit has geological rather than statistical significance In this case the lack of reliable data concerning the position of the continental margin of Greenland north of the Iceland–Greenland Ridge means that objective geometric methods cannot be used to obtain a fit In this context the large gap created by this reconstruction north of latitude 68°N is not considered as a major obstacle, because it may imply that the continental margin of Greenland in this area has subsided in a manner similar to its Norwegian counterpart

The new fit differs significantly from that of Bullard *et al*[1] in that it involves a shift of Greenland some 400 km to the south-west This brings the Tertiary volcanic province of eastern Greenland into close juxtaposition with the Faeroe Islands and on to the extension of the trend of the Faeroe–Hebridean igneous province, to which it shows both temporal and petrological links This close spatial relationship supports the suggestion implicit in Holmes'[9] original definition of the Brito-Arctic Province the rocks are derived from a common source not directly related to other volcanism along the North Atlantic Ridge system If Iceland is the surface expression of a mantle plume or 'hot spot'[10-12] which has maintained a fixed position on the Mid-Atlantic Ridge throughout the last 60 Myr (ref 13) then the traces of the Iceland–Faeroe and Iceland–Greenland Aseismic Ridges should define the relative movement of the continental masses, and any predrift fit constructed on this basis would, necessarily, place the Faeroes opposite the Kangerdlugssuag region of Greenland

Mesozoic stratigraphy and tectonic patterns in eastern Greenland[14] and north-western Europe show remarkable similarities[15], and the trend of the North Sea Graben, well defined by the major petroleum finds of the area, is coincident with the trend of the Mesozoic graben structure of eastern Greenland (Fig 2) Although the thickness[18] of sediment in the North Sea (up to 8 km) is considerably greater than in Greenland, much of this is of Tertiary age and the thickness of Mesozoic sediment (approximately 4 km) is more closely comparable to that of Greenland If these structures form a truly continuous belt then they cut across the subsequent rift system of the North Atlantic, and the view that the Mesozoic and Tertiary basins of north-western Europe are, in a simple way, related to this incipient rift must be examined more closely[17-19]

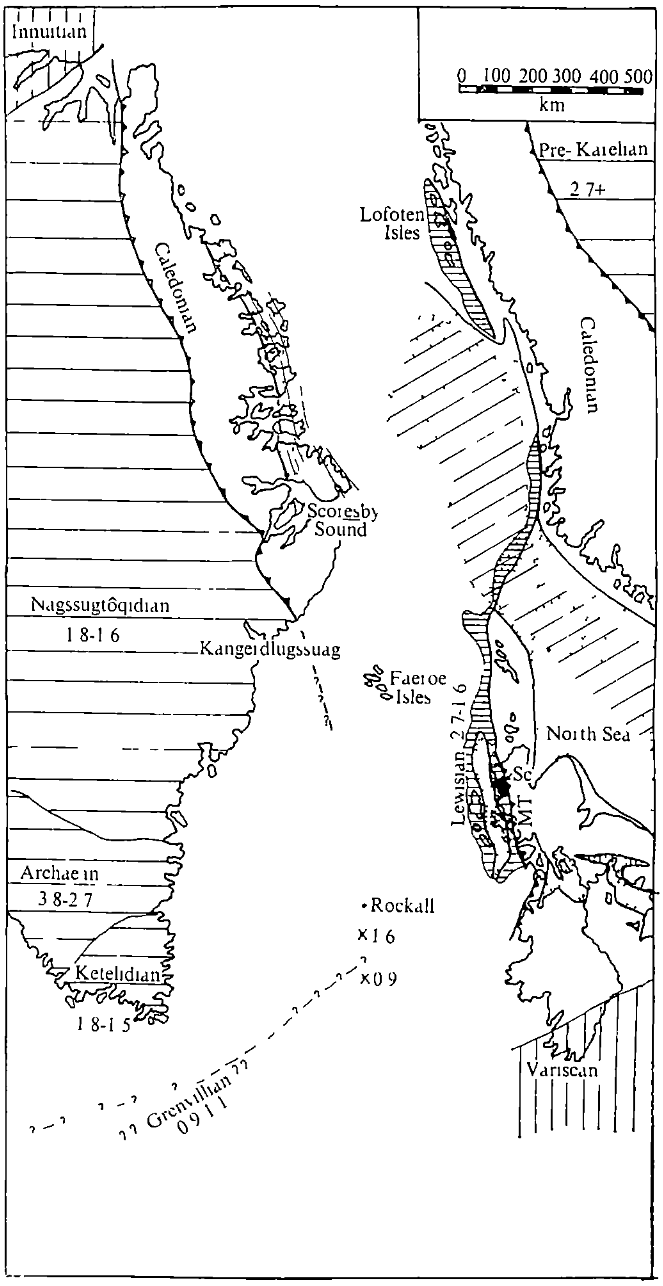

Fig 2 Major geological features of Greenland and north-western Europe Horizontal ruling, pre Caledonian orogenic belts showing radiometric ages (×10⁹ yr), vertical ruling, post-Caledonian orogenic belts, stipple, Mesozoic basins, Sc, Scourian, MT, Moine Thrust, ×, drill sites on the Rockall Plateau, with radiometric ages (×10⁹ yr)

The difficulty of aligning the Caledonian Front of Greenland[14] with the Moine Thrust Zone of north-western Scotland has been cited as criticism of the Bullard fit[20] But this was overcome to a large extent, by Bott and Watts[21], whose reconstruction produced a reasonable alignment of these features The present model, however, is not compatible with the alignment of these two structures (Fig 2) An examination of the geophysical evidence concerning the north-eastward extension of the Precambrian basement of north-western Scotland[22,23,8] strongly suggests that it takes the form of a discontinuous basement ridge trending NNE and eventually reappearing in the Lofoten Islands The basement complex of the Lofoten Islands has a radiometric history similar to that of the Laxfordian of Scotland[24,25] The published ages of rocks drilled from the Rockall Plateau[26,27] do little to clarify the position of the southerly continuation of the Caledonian Front from Greenland The date of 989 ± 5 Myr from one of the samples (Fig 2), assumed to be Grenvillian, is, however, similar to dates from immediately south-west of the Caledonian Front in Greenland[28]

The suggestion that the Precambrian histories of Scotland and Greenland are directly comparable and that the Scourian is the lateral equivalent of the Archaean of Greenland[29] requires further corroboration in view of the fact that these two regions must, on the reconstruction presented here, be assumed to be separated by the western half of the Caledonian Mountain Belt

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The Burtle Beds of Somerset—glacial or marine?

KELLAWAY[1] described the Burtle Beds as "residual masses of glacial sand and gravel ('Burtle Beds'), (which) rise out of the marshes at Catcott Burtle, Weston Zoyland and Othery" Kidson and Haynes[2] supported the earlier view of Bulleid and Jackson[3] that the deposits are of marine origin and of interglacial rather than glacial age and provenance They concluded that "any suggestion that the Burtle Beds are ice transported is quite untenable A fluvioglacial origin is so unlikely as to be unacceptable "

As evidence for a marine origin Kidson and Haynes showed that the microfauna indicate a transgressive estuarine–marine phase The sands and gravels rest conformably on clays of similar age and origin which were clearly laid down in the same transgression The clays in turn rest on a hard fissile clay interpreted as Lower Lias Hawkins and Kellaway[4], in reply, found "nothing to convince us of the marine origin of the Burtle Beds or to change our opinions of the older glaciations" They suggested that the clay beneath the sand described by Kidson and Haynes was "remoulded Lower Lias clay contaminated with microfossils or ground up shell debris,

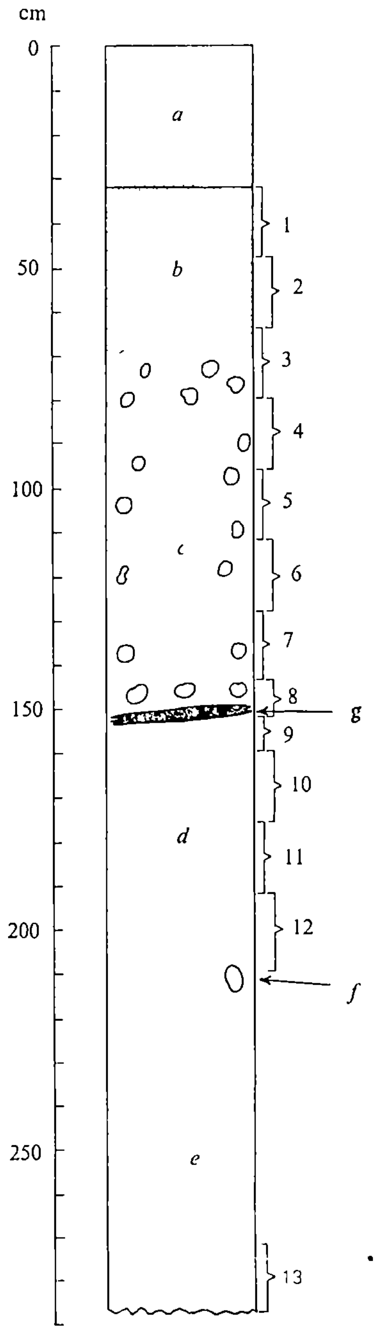

Fig 1 The section exposed in a pit at Hill Farm, Catcott Burtle *a*, Sandy soil, *b*, sand, *c*, brown clay with stones and occasional shells, *d*, hard fissile blue yellow clay, *e*, hard fissile blue clay, *f*, *Paltechioceras elicitum* (ammonite), *g*, *Corylus* (recumbent branch?) Numbers refer to samples (see Fig 2)

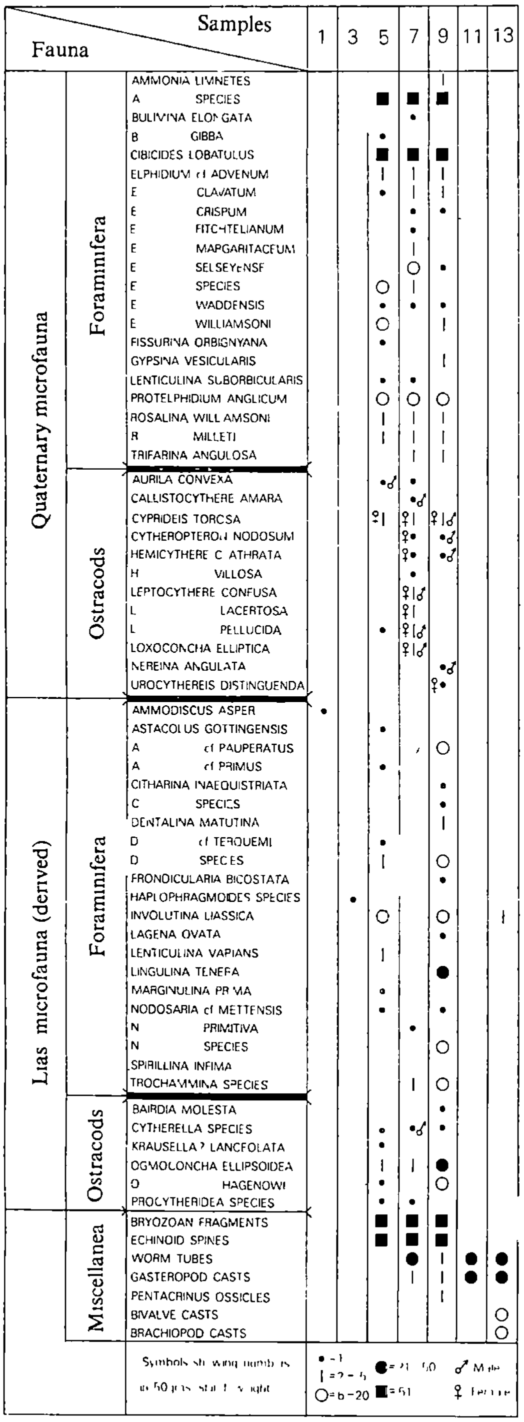

Fig 2 The distribution of fauna in the samples from Hill Farm, Catcott

some having been carried by water draining from the overlying Burtle Beds in the boreholes"

To test this argument, we dug a pit with a mechanical excavator so that samples could be obtained from a clean face where any possibility of contamination could be ruled out The pit was sited midway between boreholes 8D and 8E (Fig 2 of ref 2) at Hill Farm, Catcott Burtle (ST399430) The stratigraphy revealed is shown in Fig 1 At the passage between the two clays was a branch, root or trunk of *Corylus* (hazel), not in a growth position, which yielded a ^{14}C date of 4,280±70 b p (HAR 463) The face was sampled as indicated in Fig 1, and alternate samples were examined for microfauna (Fig 2) The results clearly confirm previous observations[2] first, that the microfauna in the Burtle clay (the upper brown clay) is a mixture of Quaternary and Liassic species with other time periods apparently unrepresented, second that there are no exclusively cold water Quaternary species, and finally that the overall aspect is consistent with estuarine accumulation The samples were also examined for pollen, but it has proved impossible to date this or other 'Burtle Bed' deposits by pollen analysis

When the distribution chart is considered in detail and compared with that for the boreholes previously examined (Fig 3 of ref 2) the close similarity of the microfaunas, particularly with 8D, is apparent, allowing for name changes in some of the ostracods, because of generic shifts[5] [6]

The deposit from which the name 'Burtle Beds' was derived is thus clearly seen at Catcott Burtle to be of estuarine–marine origin and to include Quaternary clays beneath the sands and gravels, as suggested earlier[2]

A completely new element has appeared, however, because of the discovery of *Corylus* in the pit at Hill Farm The wood occurs immediately above the passage horizon represented by sample 9 (Fig 1) If the Burtle clays were of Pleistocene age, an infinite ^{14}C age could be expected The Holocene date can only mean, however, that the overlying deposits were emplaced in the late Holocene A sand–intertidal silt relationship, similar to that at Hill Farm, is evident in an excavation for a new road bridge near Penzoy Farm (ST337349), (D D Gilbertson, personal communication) A sample of wood from Penzoy Farm is now being dated, but preliminary results suggest a similar Holocene age An examination of the altitude of the wood samples at Hill Farm and Penzoy Farm (3 5 m OD), which may be either *in situ* or driftwood, in relation to Flandrian sealevel curves for the area[7] suggests strongly that these sites were coastal towards the end of that transgression They therefore cannot now be regarded as typical Burtle bed sites It is possible that these sites are of interglacial age, and were modified in post-glacial times It is interesting to note, in retrospect, that Ussher[8] discussed and rejected the possibility of a Flandrian age for the beds

The division into a sand layer, and an underlying clay bed of similar age, arrived at on the basis of borehole evidence, has been demonstrated in an open face at Hill Farm, Catcott Burtle, eliminating the argument about contamination A comparable division was revealed in a pit at Greylake There can be no doubt about the Interglacial age of these deposits If the sands and gravels at Catcott Burtle were originally of true Burtle Bed type comparable to those at Greylake, they must have been reworked, at least on the margins, by the Flandrian sea The microfauna includes forms[2] that are known from the Flandrian and Recent of Great Britain and also occur in the last interglacial (Ipswichian) The Catcott Burtle fauna does, however, include northern forms, *Elphidium clavatum* and *Hemicythere clathrata,* and a southern form *E fitchtelianum,* which lends slight support to the idea of reworking On the other hand these deposits may be truly of Flandrian age and unrelated to what are now called Burtle Beds elsewhere in the levels

These conclusions do not call into question the age or origin of the mass of the Burtle Beds No evidence has yet been produced on which to justify a glacial or fluvioglacial origin for them All the available evidence[2], including the pre-Flandrian shell dates from Mollusca from the Burtle sands and gravels south of the Poldens[9] points to a marine interglacial, probably Ipswichian, origin The problem remains of separating the undisturbed deposits of Burtle sands and gravels from the deposits of the Flandrian sea or from earlier marine beds reworked by that sea That now seems to have washed not only islands and peninsulas of Lias and Trias, but also of Burtle sands and gravels On the basis of height alone, most of the sand batches north of the Poldens and perhaps many of those to the south must be re-examined More detailed study is called for than has yet been attempted A beginning is being made with a detailed re-examination of the deposits of Greylake

Dr R C Whatley identified the ostracods, and the results were discussed with Dr Alan Lord (University College London), Dr C G Adams (British Museum, Natural History) and Mr Ben Johnson (Arco Petroleum Co) kindly examined and commented on the Liassic foraminiferal fauna

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Dating of the rock succession containing fossil hominids at East Rudolf, Kenya

THE geology of the large complex faulted depression between the Ethiopian and Kenyan rifted, upwarped domes, is virtually unknown in detail Up until 1969 there had been only a few isolated expeditions[1-4] into the region, but since then there has been an increased amount of research around Lake Rudolf, particularly by Kenyan, American and French based workers[4] It has been shown that the sparsely inhabited semi-desert area to the east of Lake Rudolf is exceptionally rich in the remains of fossil man, and there is abundant evidence of his activities[5-28] informative hominid assemblages have been found there together with possibly the oldest known archaeological traces of hominid activity

The Plio-Pleistocene rock succession within the East Rudolf sedimentary basin contains several prominent felsic volcanic tuffs The more extensive of these tuff horizons are vitally important for both stratigraphical subdivision and relationships[20], and the dating of fossil faunas within the sequence At an early stage of the exploratory work at East Rudolf we were able to obtain a date of 2 61 ±0 26 Myr for the important KBS tuff horizon[7], using argon-40/argon-39 age spectrum analysis of a collection of large fresh sanidine crystals which had been separated from KBS pumice cobbles in the field Dating of the other tuff horizons was, however, more difficult

Certain depositional and other characteristics of the East Rudolf tuffs suggest that the base of each major tuff horizon can be regarded as an isochronous surface within rather narrow limits Thus, by mapping dated tuffs and their lateral equivalents and considering information from the geomagnetic reversal stratigraphy[22] it should be possible to produce a detailed geochronological map of East Rudolf, from which the age of any faunal or archaeological site can be estimated with close precision

A complex of Miocene–Recent volcanics and sediments borders the East Rudolf Basin on its entire eastern side The complex is cut by the main Pleistocene rift which runs between the Sugata Valley and Lake Stephanie A number of detailed studies within this area will be necessary in order to identify the provenance of the sediments and tuffs in the East Rudolf Basin and to resolve questions about the location of the ancient drainage channels and the character and timing of the source eruptions

There is wide interest in the fossil hominids from East Rudolf, the cranium KNM-ER 1470 has attracted particular attention It is, therefore, appropriate to discuss summarily the main conclusion of our 1970–1973 dating programme, although full publication of the results of these and other projects must await the completion of the field mapping

Over one hundred rock and mineral samples from East Rudolf have been analysed by a combination of either conventional K–Ar, total degassing and spectrum ^{40}Ar/^{39}Ar age or fission track dating methods[29-35] Figure 1 summarises our present conclusions regarding the East Rudolf time sequence, and illustrates the relationship between the dated tuffs and the world wide geomagnetic polarity reversal time scale The relationship of hominid fossil sites to the dated tuffs has been summarised by Brock and Isaac[22]

Dating work on a sanidine concentrate from a single pumic lump taken from the Chari Tuff, at Ileret (Fig 1)

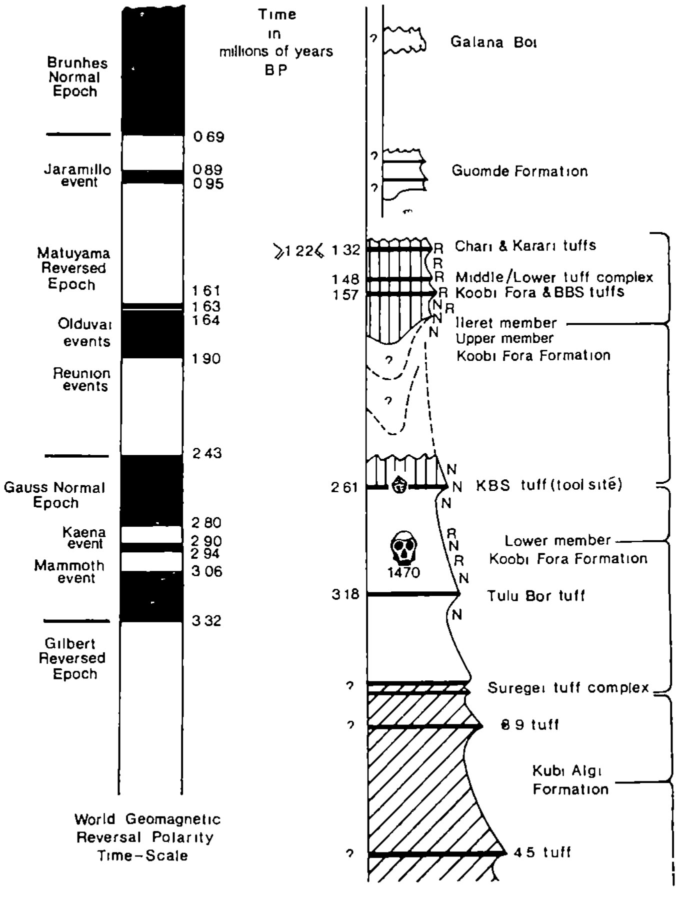

Fig 1 Dated tuffs in the East Rudolf sedimentary basin Palaeomagnetic data from Brock and Isaac[22] and stratigraphical nomenclatuure from Bowen and Vondra[20]

suggested minimum apparent ages of 1 2 Myr from total degassing analysis, and 1 28±0 23 Myr from age spectrum analysis Age spectrum analysis of another sanidine concentrate revealed minor Ar loss and excess Ar contamination effects, and indicated a minimum apparent age of 1 22±0 01 Myr for the major juvenile sanidine component

An apparent age of 1 48±0 17 Myr was obtained by total degassing analysis of sanidine concentrates from pumice taken from the Lower–Middle Tuff Complex, at Ileret (Fig 1)

Apparent ages obtained by total degassing analysis from seven independent sanidine concentrates from the Karari Tuff (Fig 1) ranged from 0 6 to 1 39 Myr Age spectrum analysis revealed that both argon loss and excess argon errors are present in these concentrates A close maximum apparent age of 1 32±0 10 Myr was obtained for the juvenile sanidine component by age spectrum analysis

During age measurements of the Koobi Fora Tuff (Fig 1) it was difficult to avoid the effects of partial and complete overprinting by later events, and the effect of contamination by xenolithic and/or detrital material Imprecise age spectrum results at first suggested an apparent age of 1 1± 1 7 Myr, whereas total degassing apparent ages, obtained from a large number of independent samples, ranged from 1 53 to 1 69 Myr Further age spectrum studies indicated Ar loss and excess Ar errors, a minimum apparent age of 1 48±0 23 Myr, and a close apparent age of 1 57±0 00 Myr for the major juvenile sanidine component Evidence of severe overprinting at around 1 0 Myr was also obtained

Apparent ages ranging from 0 87 to 1 66 Myr were obtained from the BBS Tuff Complex (Fig 1) by total degassing analysis of seven independent sanidine concentrates from pumice Age spectrum analysis revealed both Ar loss and contamination errors The two principal feldspar components in the sample analysed using this method, have apparent ages of 1 70±0 04 Myr and 1 56±0 02 Myr, respectively

A considerable amount of dating work has been done on samples from the KBS tuff (Fig 1) Although the sanidine component in this tuff has certainly been subjected to a number of partial or complete overprinting events, severe contamination or excess argon effects have not been identified Total degassing apparent ages from ten independent sanidine concentrates range between 0 52 and 2 64 Myr Seven age spectrum analyses of further, independent KBS sanidine concentrates identified two age components crystallisation at an apparent age of 2 61± 0 26 Myr with a minimum apparent age of 2 54±0 53 Myr, and overprinting by later events at about 2 42±0 02 Myr, 2 01±0 03 Myr, 1 75 Myr and 1 0 Myr (all apparent ages)

Age spectrum analysis of a sanidine concentrate from Tulu Bor pumice (Fig 1) revealed it to be a mixture of accessory sanidine with an apparent age of 4 0±0 01 Myr, and juvenile sanidine with an apparent age of 3 18±0 09 Myr

Two tuffs in the Kubi Algi Formation (Fig 1) were examined using a number of dating methods So far, however, only very imprecise indications of apparent age have been obtained These are about 3 9 and 4 5 Myr, respectively

Plio-Pleistocene plateau basalts capping the volcanic terrain north-east of the sedimentary basin have an average K–Ar minimum apparent age of 2 20±0 04 Myr The Kokoi basalts occurring within the basin have been dated using K–Ar methods, at an average apparent age of 3 62±0 36 Myr Similar basalts entering the basin from the south-east near Kubi Algi have average apparent K–Ar ages of 3 8±0 4 Myr and 3 80±0 38 Myr

Miocene peralkaline rhyolites at Kubi Algi have average K–Ar apparent ages of 7 5±0 8 Myr An obsidian from Shin has been dated using the K–Ar method, at an apparent age of 11 8±0 9 Myr A variety of basalts exposed in the area of the Suregei Cuesta have average K–Ar apparent ages between 11 6±0 5 Myr and 14 1±1 4 Myr Several extensive eutaxitic felsic ignimbrites found in the Ethiopia–Kenya border area, near Gum Dura, have average K–Ar apparent ages near to 16 15±0 12 Myr The oldest basalts which have been dated, have average K–Ar apparent ages around 17 3±1 11 Myr, and outcrop in the Buluk Gap and near Fora water hole

This dating work on rocks from the East Rudolf Basin, completed in 1973, confirms the suggested age of 2 61±0 26 Myr reported for the KBS tuff in 1970 (ref 7), and places it securely within a chronologically satisfactory sequence of dated tuffs Our conclusions are compatible with palaeomagnetic polarity reversal results from sediments and tuffs in the basin[22] The compatibility of independent evidence is a very strong argument for accepting the chronology now proposed for East Rudolf A discrepancy between this chronology and the middle part of that proposed by Brown and Lajoie[36] for the Shungara Formation of the Omo Valley of southern Ethiopia is suggested by detailed faunal correlation between the two areas[37] This is under joint investigation Important episodes of hydrothermal overprinting affected the rocks of the East Rudolf Basin between 2 05 Myr and 1 75 Myr age and again at about 1 1 Myr and 0 5 Myr age We believe that these overprints have been caused by thermal activity related to magmatic doming, major rift movements and concomitant volcanism It is significant that the three major rift faulting events in Kenya and Tanzania have been dated at 2 1 Myr, 1 2 Myr and near to 0 4 Myr, respectively[38]

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Probing broad lines in molecular spectra by optical site selection spectroscopy

WE report evidence that suggests that narrow-line spectra can be obtained for many organic compounds without the difficulties commonly found with traditional mixed-crystal line-narrowing techniques Furthermore, our findings suggest a new method by which low temperature line-broadening mechanisms may be studied

Even at very low temperatures the optical absorption and fluorescence bandwidths of many organic compounds in solution remain very broad, typically several hundred cm^{-1}, with unresolved or poorly resolved structure The techniques of mixed crystal spectroscopy[1,2] and its more recent variant due to Shpol'skii[3] have been developed to probe whatever structure may be hidden in these broad lines or bands (The spectra of neat crystals often yield sharp lines at helium temperatures but these spectra are often more representative of collective excitations or excitons and not the 'unperturbed' molecular spectrum) Unfortunately, narrow-line mixed-crystal and Shpol'skii spectra can be obtained only in those special instances in which a suitable host crystal or quasi-crystal can be made to fit the subject molecule The purpose of the host is to hold every subject molecule in the sample in one or another of a small number of available environments (crystal fields) For most organic compounds suitable hosts are not known

The method for obtaining narrow-line spectra which we describe here was first demonstrated by Szabo[4], in a study of ruby We conclude from studies of a variety of organic compounds that the method is indeed useful in obtaining high resolution spectra of organic molecules in low temperature solutions It seems to be much more flexible than the mixed-crystal and Shpol'skii methods because is discards the very special host matrices on which those techniques are based and can be used with a variety of hosts including glasses and polymers

It seems probable that the vibronic lines of two identical molecules in a frozen amorphous host solution are shifted with respect to each other if and when their local environments differ, and that in many host media the shift may far exceed the widths of the individual vibronic lines Furthermore, it is a natural assumption that a molecule interacts optically only with light that is resonant with one of its vibronic lines

If these assertions are approximately true, then it should be possible to do 'mixed-crystal' spectroscopy in arbitrary vitreous hosts simply by using optical probes that are spectrally very narrow, because the light itself will pick out only those subject molecules that happen to be in identical local environments or sites in the host For convenience we will term this technique 'optical site selection spectroscopy'

We have observed, from a variety of organic compounds, narrow-line fluorescence similar to that reported by Personov *et al*, for perylene[5] In every case we used spectrally narrow ($\Delta\nu/c$<0 2 cm^{-1}) laser excitation An example of our results is shown in Fig 1 When naphthacene in a frozen vitreous solvent is excited by a conventional $\Delta\nu/c$ ~ 1,000 cm^{-1}) source, the result is the normal emission spectrum (Fig 1*a*) The vibrational bands, although well resolved, are broad, about 230 cm^{-1} for the 0 0 band Excited in its 0 0 band at 4,765 Å by an argon ion laser, however, naphthacene exhibits the strikingly different spectrum (Fig 1*b*) The emission line that occurs at about 4,830 Å is 3 cm^{-1} wide The various sharp lines in Fig 1*b* are allowed transitions from the lowest level of the excited state to the vibrational levels of the ground state The broad band accompanying each line can be interpreted as the analogue of the phonon sidebands often observed in mixed-crystal spectra[6] As a check, a vibrational analysis of the spectrum in Fig 1*b* was carried out The ground state vibrational frequencies thus obtained are in excellent agreement with those obtained for naphthacene by different methods[7]

According to these results it seems natural to conclude that, at low temperatures under broadband excitation, naphthacene exhibits an inhomogeneously broadened

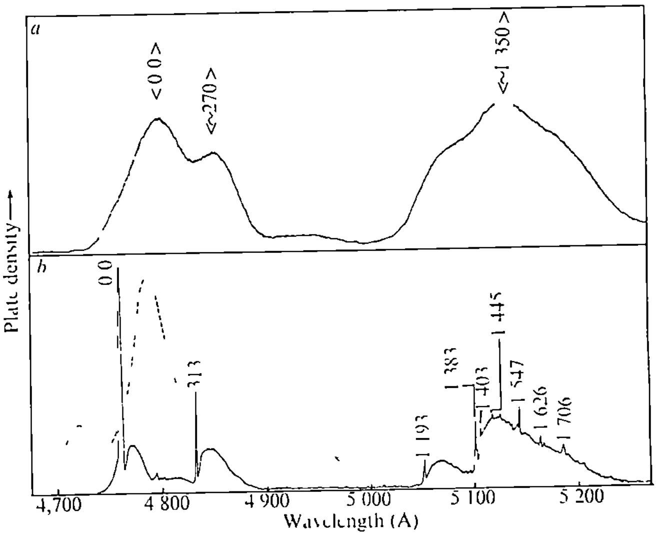

Fig 1 The fluorescence spectrum of a 10^{-4} M solution of naphthacene in a 2-methyltetrahydrofuran glass at 4 2 K *a*, Excited with broadband (~ 100 Å) Hg line at 4,358 Å, *b*, excited with 4,765 Å argon ion laser line (bandwidth < 0 2 cm^{-1}) Dotted curve is a portion of the absorption spectrum of naphthacene at 4 2 K The 0 0 bands are taken as the origins The distances to succeeding peaks are given in cm^{-1}

spectrum, one that is simply the superposition of many sharp spectra such as the spectrum in Fig 1*b* There is thus no intrinsic importance in either the width or the absolute location of the peaks in Fig 1*a*, since they merely reflect the wide variety of environments provided for naphthacene by the particular vitreous host used For this reason we have used brackets ⟨ ⟩ to signify an average value in labelling the principal peak in Fig 1*a*

If our interpretation of the origin of low temperature spectra is valid, excitation by very narrow band light in higher frequency absorption bands instead of the 0 0 band could lead to a much greater number of fluorescence lines (which may be resolvable under the appropriate conditions)

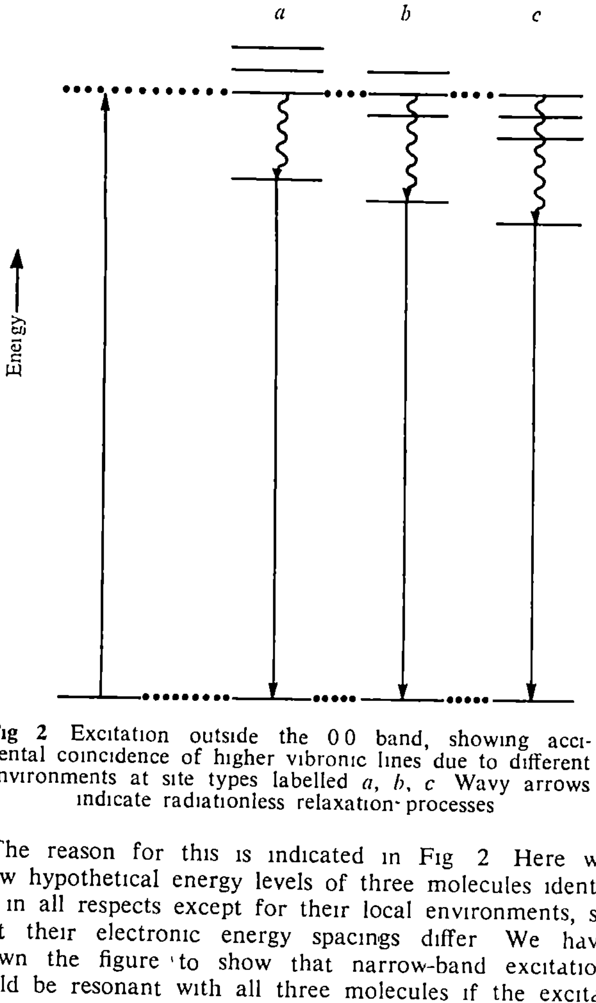

Fig 2 Excitation outside the 0 0 band, showing accidental coincidence of higher vibronic lines due to different environments at site types labelled *a*, *b*, *c* Wavy arrows indicate radiationless relaxation processes

The reason for this is indicated in Fig 2 Here we show hypothetical energy levels of three molecules identical in all respects except for their local environments, so that their electronic energy spacings differ We have drawn the figure to show that narrow-band excitation could be resonant with all three molecules if the excitation were at the frequency shown That is, excitation higher than the 0 0 band may lead to accidental coincidences and cause the fluorescence spectrum to be characteristic of several or many (in this example, three) environments at once

Phenanthrene in methylcyclohexane typifies the multiple-line phenomenon In Fig 3 we show the result of very narrow-band excitation of phenanthrene at 3,250 Å, about 0 24 eV (220 Å) above the centre of the ⟨0 0⟩ absorption band The spectrum does contain narrow lines, and they are more numerous than previously observed in mixed-crystal fluorescence spectra of phenanthrene[3] The increased background is probably due to a superposition of the 'phonon' bands associated with all of the extra lines

Studies of substituent effects on narrow-line spectra were also carried out for derivatives of two organic compounds, naphthacene and anthracene In two parent aromatic hydrocarbons and four derivatives the spectra ranged from very broad and unresolved to very narrow lines In particular, the broadening effect of phenyl groups, often observed at room temperature, remains substantial at 4 K even with laser excitation Methyl groups were also found to broaden the spectra, whereas halogen substitution had no apparent effect

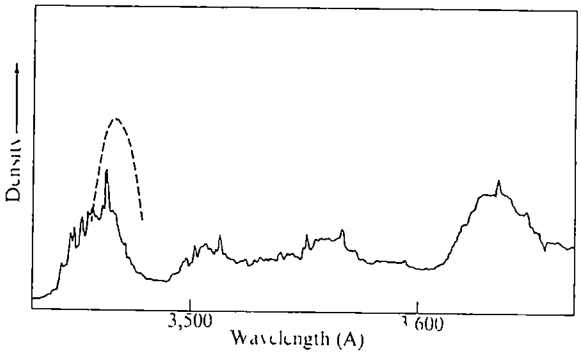

Fig 3 The fluorescence spectrum of a 10^{-4} M solution of phenanthrene in methylcyclohexane glass at 4 2 K Excitation is at 3,250 Å with a He–Cd ion laser The excitation is 0 24 eV (220 Å) above the ⟨0 0⟩ band The dotted curve is a portion of the ⟨0 0⟩ absorption band of phenanthrene in methylcyclohexane glass at 4 2 K

We also examined a number of organic dyes Although only broadband emission was obtained from ionic dyes, a few narrow lines were observed in a merocyanine

We have also studied solvent effects in the production of narrow line spectra, including the effects of solvent polarity Such compounds as naphthacene are only slightly sensitive to the polarity of the host solvent The linewidth of the 313 cm^{-1} naphthacene fluorescence line is about 3 cm^{-1} in nonpolar methylcyclohexane, and about 5 cm^{-1} in the polar mixture of ethanol and methanol (1 1) Personov indicated a similar weak effect for perylene[5] In azulene, which changes its dipole moment by 1 1 debye on excitation[9], polar solvent broadening is, however, severe

In summary, optical site selection in low temperature glasses provides a straightforward technique for observing vibronic structure normally obscured by inhomogeneous broadening Typical inhomogeneous linewidths in these experiments are on the order of several hundred cm^{-1} The homogeneously broadened 'zero-phonon' lines observed are about 3–10 cm^{-1} wide Their measurement may prove to be a sensitive probe of the local solvent environment To the extent that a low temperature glass represents a liquid solution at a fixed instant in time, studies of this nature may also provide insights into broadening mechanisms in liquid solutions

We have begun a detailed examination of some of these effects using tunable laser excitation

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The origin of the far-infrared polarisation of liquid water

LIQUID water has a strong far-infrared polarisation The origin of this has rarely been discussed in detail, although it causes the difference between the optical permittivity of about 1 8, and the limiting high frequency microwave (ε_∞) permittivity of about 4 2 The phenomenon has been attributed by Zafar *et al*[1] to a high frequency relaxation of amplitude 2 4 in the permittivity, and a relaxation time of ~50 ns resulting from the orientation of water molecules that are free of hydrogen bonds Unfortunately, the authors did not apply Sherlock Holmes'[2] adage that when you have eliminated the impossible, whatever remains, however improbable, must be the truth Not all possible explanations of this polarisation have been considered and eliminated, although the correct one has been mentioned There can be little doubt that there is, at present, no evidence for a new orientational relaxation

Another possible cause of the polarisation is, of course, resonance absorption, discussed briefly by Zafar *et al*[1] in terms of a single damped resonance absorption centred at 170 cm^{-1} It may not be possible to describe, however, a resonance absorption band using a single damped resonator, there may be a continuous distribution of resonators, and any arbitrary distribution of intensity may occur, even one that mimics a relaxation absorption Relaxation and broad resonance absorptions cannot be distinguished by direct measurement, and so, to decide which is occurring in liquid water, the spectrum of water can be compared with that of ice, which has no relaxation absorption in this frequency region

Ice I has an infrared polarisation responsible for the difference between the optical and microwave dielectric constants 1 8 and 3 1, respectively[3] The difference, Δn, between the corresponding refractive indices is proportional to the negative second moment of the absorption spectrum

$$\Delta n = \tfrac{1}{2}\pi^{-2} \int_{\text{band}} K(\nu)\, \nu^{-2} d\nu$$

where $K(\nu)$ is the absorptivity at wave number ν In ice, the main contributory bands are the H_2O internal motions in the range 3,600–1,000 cm^{-1}, the H_2O rotational vibrations centred in the infrared at 850 cm^{-1}, and the H_2O translational vibrations centred at 229 cm^{-1} They contribute, respectively 0 0506, 0 0728 and 0 347 to the infrared component of the low frequency refractive index[3]

Internal motions contribute little in ice, they contribute even less in water, because the O–H stretching frequencies are higher They might contribute, say 0 05 to the refractive index The rotational vibrations in ice extend from 525 to 1,040 cm^{-1}, the infrared intensity being centred at ~850 cm^{-1} The effective dipole moment is 1 45 *D* (ref 4), compared with the value for the vapour of 1 84 *D* In the liquid there is a broad band because of these vibrations Its peak is shifted from 850 cm^{-1}, the frequency in ice, to about 650 cm^{-1}, and it is greatly broadened, the lower limit decreasing from 525 cm^{-1} to 300 cm^{-1} or less The negative second moment is, therefore, about twice as great for the liquid as for ice, and so its contribution to the low frequency refractive index is about 0 15 A more accurate value may be obtainable from the measured spectrum, but it would be difficult to separate the overlapping rotational and translational bands with any reasonable certainty

Absorption in ice, which results from the translational vibrations, extends from 318 cm^{-1} to zero frequency[5] and its maximum is at about 229 cm^{-1} The integrated intensity[6] shows that the effective charge for the stretching of the hydrogen bond is about 0 4*e*, where *e* is the electronic charge

The band is broadened and the peak shifted to about 220 cm^{-1} in vitreous ice[7], and undoubtedly persists in the liquid, because the diffusion coefficient, the viscosity, and the relaxation of molecular and nuclear orientational polarisation[8] indicate that a water molecule requires about 10 ps to change its position or orientation at 25° C, whereas a frequency as low as even 30 cm^{-1} corresponds to about 1 vibration ps^{-1} It is no doubt broadened greatly by the increased disorder of the liquid as compared with ice and by the short lifetime of a state In addition, the stretching force constant of the hydrogen bonds undoubtedly takes on a range of values because of the range of hydrogen bonds All of these processes broaden the band and increase its negative second moment Because of the increased length of the hydrogen bond, the force constants are undoubtedly less in water than in ice, and that will cause the whole band to shift to low frequency, and will further increase the second moment The band has not been analysed in detail, but the peak intensity is at about 170 cm^{-1} (see ref 9 and refs quoted there) If the whole ice band were shifted by the ratio 170 229, the negative second moment would be doubled, and the contributions to the low frequency refractive index would be doubled to about 0 7 The discussed broadening would further increase the contribution The increased length of the hydrogen bond might then decrease the effective charge, thus reducing the contribution to the refractive index

It seems likely that the internal rotational and translational resonance absorption contribute somewhat less than about 0 9 to the low frequency refractive index, which corresponds to a value for ε_∞ of somewhat less than about 5, compared with the experimental value of 4 2

It seems, therefore, that there is little or no room for a relaxation absorption, and that if it does occur, it is at least an order of magnitude less than that assumed by Zafar *et al*[1], and is strongly overlapped by resonance absorption

This conclusion is confirmed by the H_2O–D_2O isotope effect in the spectrum If there were a relaxation absorption because of the rotation of molecules, the relaxation times for D_2O and H_2O would have an approximate ratio of 1 4 The relaxation times deduced by Zafar *et al* are in the ratio 0 85 Absorption because of damped translational vibrations, for which corresponding features would be at frequencies and absorptivities in the ratio 1 05, is more consistent with the measurements summarised by Zafar *et al*[1] when allowance is made for the effects of the well established orientational relaxation Both the infrared and Raman spectra of H_2O and D_2O have bands centred at about 170 cm^{-1}, and there is a Raman band at about 60 cm^{-1} (ref 10) These no doubt result from damped translational vibrations, not from rotational motions

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DRS HASTED, ZAFAR AND CHAMBERLAIN REPLY Although he has cast us[1] in the role of Inspector Lestrade[2], Dr Whalley[3] performs a useful service by questioning our neglect of resonance absorptions in the discussion of the enhanced permittivity increment, $\varepsilon_\infty - n^2$, of water Complete analysis of the origins of this increment must await the collection and analysis of refractive index data of improved accuracy over a much wider frequency range than has so far been investigated, but in the meantime we point out that the application of the Kramers–Kronig relationship to absorption data alone, can be confusing The integrated negative second moment of the power absorption coefficient contributes proportionally not to ν^{-2} but to ν^{-1}, so that a shift of the absorption band by a factor of 229 170 alters its contribution to n^2, not by 235% but by 83% Further, the maximum of the liquid water band taken to be at 170 cm^{-1} is now placed as high as 200 cm^{-1} (ref 4)

In liquid water, both the 683 cm^{-1} and the 200 cm^{-1} band contribute to the $\Delta\varepsilon'$ increment just as they do in ice They arise from intermolecular librational resonance processes Normal coordinate analysis[4,5] has been used to assign the 683 cm^{-1} band to libration of the symmetry species B_2 of the $(H_2O)_5$ pentamer, and the 200 cm^{-1} band to the translational modes of species A_1, B_1 and B_2 Both processes are resonant rather than relaxational, and exhibit an S-shaped 'anomalous dispersion'[6] in ε' Libration processes also contribute an increment $\varepsilon' \propto \mu^2/I\nu^2$, according to Poley[7] Note that it is to ε' and not to n that there is a contribution proportional to ν^{-2} This type of absorption has been called 'Poley absorption', and is found in many unassociated organic liquids in the submillimetre region (for example ref 8) Apparently, no band corresponding to free molecules is found in water or in alcohols[9], with the exception of t-butyl alcohol, in which there seems to be less association (J C , G J Davies, J B H and M S Z , unpublished data)

We believe that there are only a small proportion of free molecules in liquid water, and that their reorientation is a relaxation rather than a resonance process The extraordinarily large bandwidth does not seem to be strongly temperature dependent, as would be the case for a resonance process, nor is the centre of the band dependent upon I, the molecular moment of inertia

There is, as yet, no evidence to rule out the presence of an underlying continuum such as may be contributed by a relaxation process, although its extent and magnitude may not be as large as would at first seem Certainly, the absence of a second relaxation in ice should not lead us to anticipate its absence in liquid water Resonance processes in the submillimetre band are often accompanied by an actual minimum in the refractive index[10], or in the permittivity function, and Poley absorption may be of this type This phenomenon may also contribute in liquid water in the 1–100 cm^{-1} range Our double Debye analysis neglects such a contribution and leads to a discrepancy in ε_∞ (ref 1, Fig 6) which could perhaps be resolved if it were taken into account

No relaxation isotope effect, $\tau(D_2O)/\tau(H_2O) = 1\ 4$, is to be expected, because the molecular moment of inertia, although involved in resonance rotation, is not involved directly in relaxation theory For example, it does not appear in the water principal relaxation as interpreted on the Debye–Stokes theory[11] The probable error on both proposed second relaxation times is very large, and the ratio could lie anywhere between 0 5 and 1 5

We do not wish to imply that the resonance processes can be neglected in comparison with the relaxation of free molecules, as could have been concluded from our ambitious attempt to construct a Cole–Cole diagram between ε_∞ and n_D^2 Rather, the increment must be a summation

$$\varepsilon_\infty - n_D^2 = \Delta\varepsilon'(\text{libration}) + \Delta\varepsilon'(\text{translation}) + \Delta\varepsilon'(\text{relaxation})$$

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Black polar bears

WHITE coloration of many animals in cold environments decreases the possibility of visual detection because of the lack of contrast between the animal and its white background White hair thus provides concealing coloration for prey species such as arctic hare (*Lepus arcticus*), and for predators such as the polar bear (*Ursus maritimus*) and arctic fox (*Alopex lagopus*)

Such animals often live in areas where aerial detection is feasible because of the lack of overhead cover, yet present remote sensing techniques do not usually distinguish these white animals against a white background Thermal infrared imagery will detect some white animals, including the polar bear[1] and white-coated pups of the grey seal (*Halichoerus grypus*) (N A Ø , and D M L , unpublished) but only in certain weather conditions

When cold and windy, sufficient temperature differences may not exist between a well insulated animal and its environment to enable detection by a thermal scanner

Our preliminary attempts to improve detection of white animals on snow suggested that photography in the near ultraviolet band (300–400 nm) of the electromagnetic spectrum markedly increased contrast between some white animals and their backgrounds[2]

We recently obtained oblique photographs of polar bears on the tundra south of Churchill, Manitoba, Canada The low visual contrast of a polar bear on snow is shown by black and white photography in the visible region of the electromagnetic spectrum (Fig 1*a*) Ultraviolet photography, however, resulted in a black image of the polar bear against white snow (Fig 1*b*)

This difference in the ultraviolet absorbance and reflectance characteristics of the polar bear pelt and snow has direct applications for remote sensing of polar bears in the field Although the distance between polar bears on the tundra makes photographic surveys impractical over wide areas of the arctic, ultraviolet photography may be useful for aerial detection and censusing bears in specific areas, where they congregate at certain times of the year, or along known migration routes The ultraviolet band may also be used in long range detection and observation of bears on ice At present, such observations, often made by telescope, are also hindered by the lack of contrast between the bear and its environment Contrast may be enhanced by coupling an ultraviolet transmitting lens covered by a visibly opaque, ultraviolet transmitting filter, with an image intensifier whose spectral sensitivity extends into the near ultraviolet region of the electromagnetic spectrum

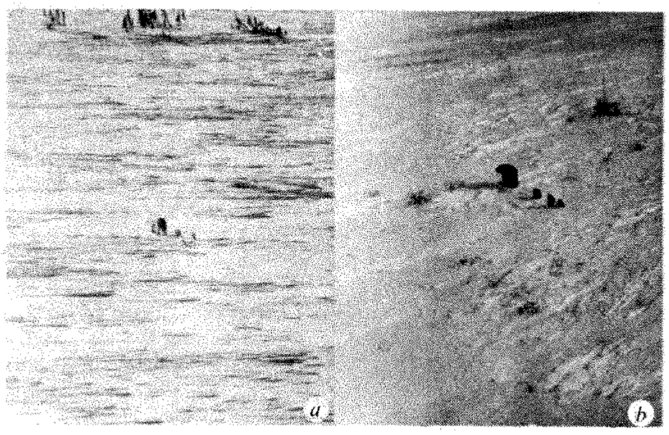

Fig. 1 Photography of a polar bear (*Ursus maritimus*) and three cubs on tundra. *a*, Normal black and white photograph in the visible portion of the electromagnetic spectrum (Kodak Double-X Aerographic 2405 film, Hasselblad 150 mm Sonnar lens, f/16, 1/500 s). *b*, Ultraviolet photograph (Kodak Aerographic Double-X 2405 film, Hasselblad 105 mm UV-Sonnar lens, Kodak 18A filter, f/5.6, 1/500 s).

This observation of 'black' polar bears seems to have further implications. At present, the biological significance of white coat coloration in animals is not clear. Whiteness in arctic climates obviously provides camouflage for many birds and mammals. Theoretical analysis and quantitative measurements suggest that some white pelts transfer solar energy more efficiently to the skin than dark pelts[3,4]. Thus white coloration may also play an important role in thermoregulation for a number of species including the white-coated pups of the harp seal (*Pagophilus groenlandicus*)[5]. The older view that dark pelts absorb solar radiation while white pelts reflect it and thus reduce radiation absorption[6] is probably incorrect. Regardless, the fact that some white pelts absorb ultraviolet radiation while others, such as the arctic hare reflect it[2], suggests that any discussion of "life's color code"[6] is incomplete without further consideration of the near ultraviolet component in solar radiation.

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Metabolic hydroxylation of a chlorobiphenyl containing only isolated unsubstituted positions—2,2′,4,4′,5,5′-hexachlorobiphenyl

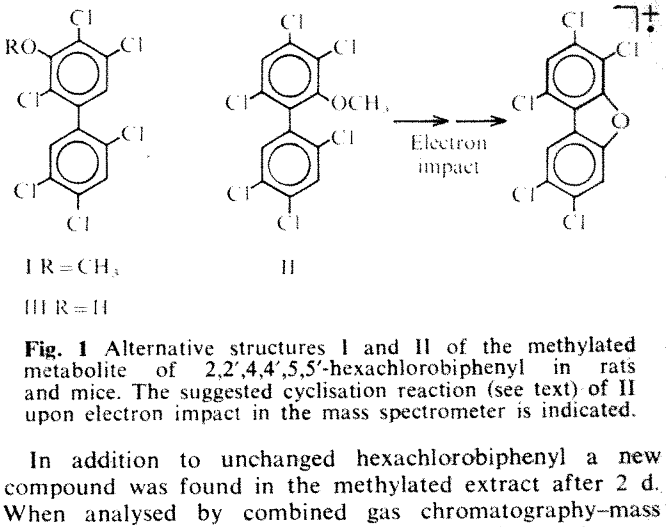
RECENT investigations on the metabolism of chlorobiphenyls of known structure have shown that compounds containing four chlorine atoms or less are hydroxylated by rats, rabbits and pigeons[1–5]. All the compounds investigated have two unsubstituted carbon atoms in ortho positions to each other. It has been suggested that this substitution pattern is required for the metabolic breakdown of chlorobiphenyls[6]. A thorough investigation of the chlorobiphenyl residues found in man and in higher animals partly confirms this suggestion[7].

Hutzinger *et al.*[2] have investigated the fate of a chlorobiphenyl containing only isolated, unsubstituted carbon atoms—2,2′,4,4′,5,5′-hexachlorobiphenyl—in rats, pigeons and trout. No hydroxy-containing metabolites were detected in the excreta by the methods used. This chlorobiphenyl is one of the major components in technical PCB with chlorine contents above 50%. Furthermore, it is found in relatively high amounts in all species investigated including man[7].

It has been shown, however, that rabbits given 1,2,3-, 1,2,4- or 1,3,5-trichlorobenzene always excreted chlorinated monophenols. The yields over 5 d were 78, 42 and 5%, respectively[8].

The fact that even 1,3,5-trichlorobenzene (containing only isolated unsubstituted carbon atoms) is hydroxylated, led us to reinvestigate the behaviour of 2,2′,4,4′,5,5′-hexachlorobiphenyl in some animal species.

Rats were given the chlorobiphenyl[9] in peanut oil (20 mg ml^{-1}) and an amount corresponding to about 15 mg was consumed during the night by each animal. Faeces were collected daily, dried and extracted with ether (Soxhlet extraction), The extracts were divided into two parts, one of which was treated with diazomethane. After evaporation of solvent the residue was dissolved in hexane and shaken with sulphuric acid before analysis by gas chromatography using an electron capture detector[7]. The acid treatment removes most unwanted extractives but does not affect chlorobiphenyls and methoxy-chlorobiphenyls.



I R=CH$_3$ II

III R=H

Fig. 1 Alternative structures I and II of the methylated metabolite of 2,2′,4,4′,5,5′-hexachlorobiphenyl in rats and mice. The suggested cyclisation reaction (see text) of II upon electron impact in the mass spectrometer is indicated.

In addition to unchanged hexachlorobiphenyl a new compound was found in the methylated extract after 2 d. When analysed by combined gas chromatography–mass spectrometry (quadropole instrument) this substance showed a molecular ion at 388 mass units (m.u.) (6 Cl) corresponding to a methoxyhexachlorobiphenyl. The structure of this compound (Fig. 1), obviously derived from a phenolic metabolite of 2,2′,4,4′,5,5′-hexachlorobiphenyl, must be I or II provided no migrations of chlorine atoms have taken place.

Mass spectral studies performed in this laboratory using about 35 synthetic methoxychlorobiphenyls (S. J. and G. S., unpublished) have shown that compounds containing a methoxy group in the two position, II, for example, readily lose CH$_3$Cl (50 m.u.) upon electron impact. This results in very abundant peaks in the range 83–234% of the molecular ion at M$^+$-50 m.u. which can be explained by the formation of cyclic structures (Fig. 1).

Similar fragmentation patterns have previously been

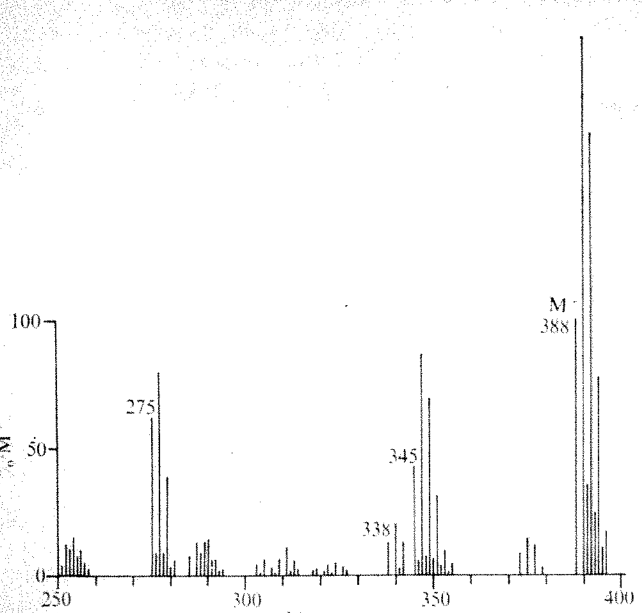


Fig. 2 Part of the mass spectrum of the methylated metabolite.

observed in the mass spectra of 2-methoxychlorodiphenyl ethers[10,11].

The loss of CH_3Cl in the mass spectra of all 3- or 4-methoxychlorobiphenyls investigated so far have on the other hand, never exceeded 30% of the molecular ion. The mass spectrum of the methyl ether of the present metabolite (Fig. 2) shows a peak at M^+-50 m.u. which is only 13% of the molecular ion. This fragmentation strongly favours structure III of the original phenol. Final proof for the structure, however, must await comparison with synthetic material of known structure.

The elimination rate of unchanged hexachlorobiphenyl and the metabolite (assuming equal detector response on GLC) is shown in Fig. 3. The total amount of metabolite excreted during 7 d was calculated to be about 1.3% of the dose. The corresponding figure for unchanged biphenyl was about 7%. No trace of III or conjugates thereof were found in the urine.

Our experiments do not discriminate between microsomal or microbial hydroxylation of the chlorobiphenyl as no bile cannulation experiments have been performed. But the presence of the metabolite in the faeces—and not in the urine—is in accordance with the results of other investigations concerning the metabolism of PCB in rats[3,4].

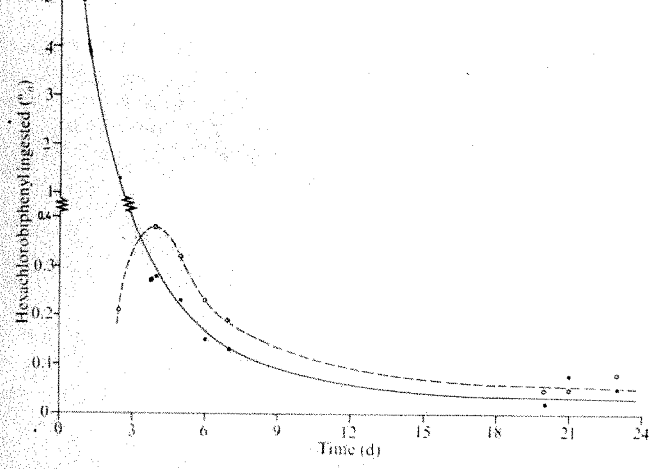


Fig. 3 Excretion rate (faeces) of 2,2′,4,4′,5,5′-hexachlorobiphenyl (———) and its metabolite (– – – –) from a rat as % hexachlorobiphenyl ingested.

Control experiments showed that bacterial action during the drying of the faeces was not responsible for the hydroxylation.

Mice given the hexachlorobiphenyl also excreted III (GLC) in the faeces. When the substance was given to quails (about 11 mg per animal) no metabolite could be detected in the excreta. It was, however, found that large amounts of unchanged chlorobiphenyl were excreted in both faeces and eggs—about 9 and 33%, respectively, during 10 d.

The results presented here show that a chlorobiphenyl containing only isolated unsubstituted positions can be metabolised by mammals albeit at a lower rate than chlorobiphenyls containing vicinal hydrogen atoms. It seems, therefore, that most of the high chlorinated compounds in PCB can be excreted not only unchanged but also as metabolites. The latter route implies that the chlorobiphenyls are not further circulated in the environment but that new compounds are released the persistence and toxicity of which are not sufficiently known.

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Sexual maturation of Japanese eel and production of eel larvae in the aquarium

THE life history of the eel is complex and despite many investigations little is known about its embryonic development. Some investigators such as Grassi and Calandriccio (1896: cited in ref. 1) and Fish[1], described the supposed early development of eels, using developing eggs collected by a trawl for young fish in suspected spawning grounds of eels. The time of fertilisation of these eggs, however, was unknown, and the species involved was uncertain. Artificial maturation of eels by hormonal treatment is another approach which has been attempted by many workers[2–6]. They all, however, failed to obtain mature eggs and sperm and thus to clarify the embryonic development of eels. Here we describe the first success with this approach.

Table 1 Early development of Japanese eel at 23° C

Time after fertilisation	Stage	Remarks
20—50 min	Single cell	Pelagic, with appearance of perivitelline space; egg diameter was 1.3 mm.
80 min—3⅓ h	Cleavage	Typical discoidal cleavage.
3¼—5½ h	Morula	Three to five layers of blastomeres.
5¼— 9 h	Blastula	Blastocoel discernible from outside.
9½—12 h	Gastrula	Blastoderm covering ¼ of yolk.
13—18 h	Embryonic body formation	Blastoderm covering ⅓ to ⅔ of yolk.
19—24 h	Blastopore closure	Differentiation of head and 3 to 12 somites.
25—27 h	Eye vesicle and ear vesicle formation	Tail bud formation.
28—33 h	Heart formation	About 32 somites.
35—37 h	Start of heart pulsation	Embryo covering entire yolk.
38—45 h	Hatching	Membranous fin well developed; no pectoral fins visible.

Silver eels of *Anguilla japonica* were caught in the Mabuchi and Hiranuma rivers in Aomori prefecture (Japan) during September 1973. They were transferred to Hakodate and raised in circulating purified seawater tanks with a capacity of 5.5 gallons. Male eels were injected with Synahorin, a mixture of pituitary gonadotrophin and chorionic gonadotrophin (Teikoku Zoki), and the females were injected with saline suspension of chum salmon pituitary as previously reported[7,8]. More than 20 males and five females became fully sexually mature following these treatments. Eggs and milt were stripped from the fish into glass dishes; fertilisation occurred, and the development of the eggs in normal seawater at 23° C was observed. The spherical ripe eggs (Fig. 1) were white and transparent; they measured about 1.0 mm in diameter. The development of the eel egg is summarised in Table 1.

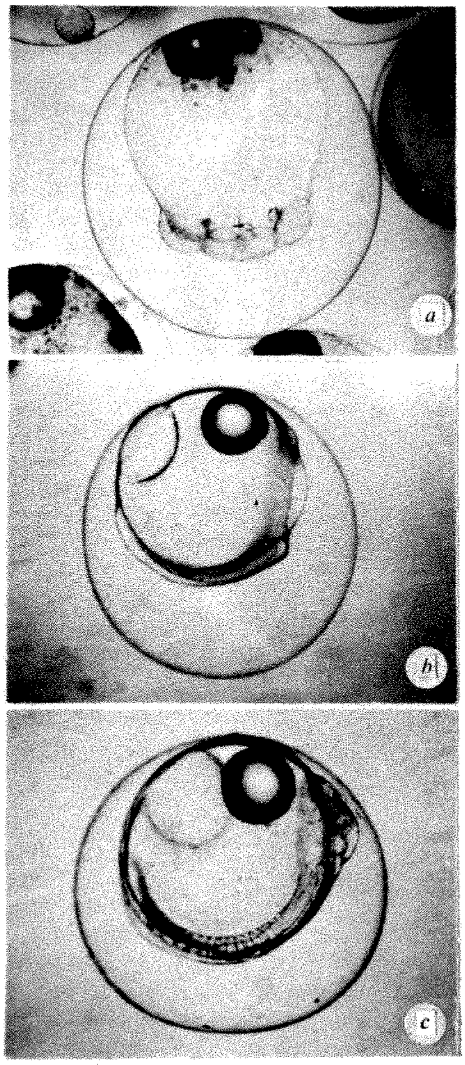

Fig. 1 Living eggs at, *a*, eight-cellstage, × 30; *b*, closing-blastopore stage, × 30; *c*, stage of eye vesicle formation, × 30.

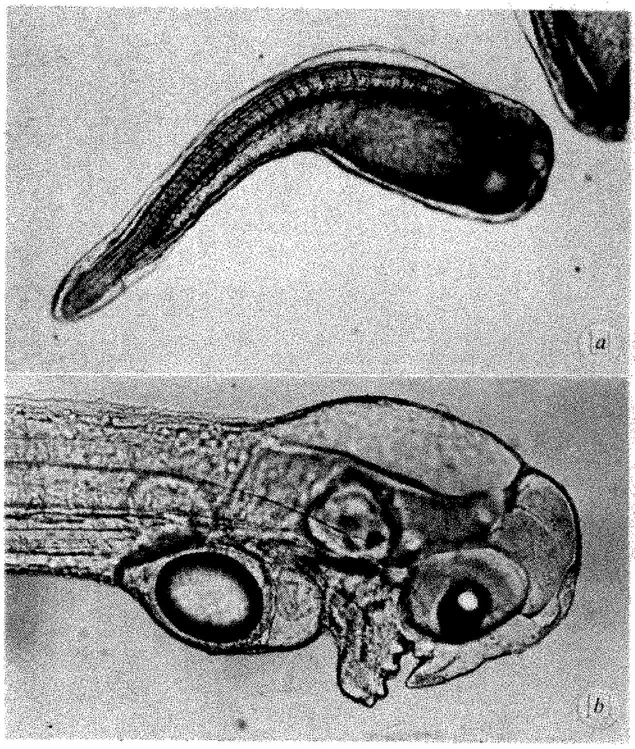

Fig. 2 Living larvae: *a*, 30 min after hatching, × 28; *b*, head of larvae, fifth day after hatching, × 70.

Hatched larvae (Fig. 2) survived for about 5 d. They measured about 4.8 mm, 5.3 mm, 5.9 mm and 6.2 mm in body length on the second, third, fourth and fifth days after hatching, respectively. By the sixth day, when the yolk sac of larvae was almost absorbed, most larvae showed curvature of the tail and then died. Some surviving larvae measured about 5.8 mm in average length. By the third day after hatching, the mouth and anus of the larvae were open, and the pectoral fins also appeared. The brain differentiated into fore, mid and afterbrain, showing a large fourth ventricle. From the fifth to sixth day, the jaw of the larvae developed conspicuously and tooth anlagen characteristic of muraenoid larvae appeared. Fifty-three pre-anal and about 48 postanal myomeres were counted in the sixth day larvae.

These observations allow us to correct and complete earlier claims.

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A long continuous pollen sequence from north-eastern Australia

FEW pollen diagrams from Australian Quaternary deposits have been published and none of these extends beyond 10,000 BP. In north-eastern Australia, Lake Euramoo[1] provides a pollen record from 9,700 BP to 1,500 BP and Quincan Crater[2] gives evidence of vegetation changes from 7,250 BP to the present day. Here I outline results from a third site on the Atherton Tableland, Lynch's Crater, which provides a much longer vegetation record.

Lynch's Crater is of volcanic origin, about 600 m in diameter and filled with organic lake and swamp deposits to 45 m below the crater rim. The crater wall has been breached and further swamp development is thereby prevented. In comparison with previously described sites (Fig. 1), Lynch's Crater receives a much higher mean annual rainfall (approximately 2,500 mm), though the crater is at a similar altitude (760 m) and is also located within the basalt-covered Tableland. Under undisturbed conditions, Lynch's Crater was surrounded by Complex Mesophyll Vine forest (terminology from ref. 3), whereas within the area as a whole, rain forest occurs on all well-drained soils with a mean annual rainfall greater than 1,400–1,500 mm. Under lower rainfalls and on soils with impeded drainage, sclerophyll vegetation, normally dominated by species of *Eucalyptus*, or *Melaleuca*, replaces rain forest.

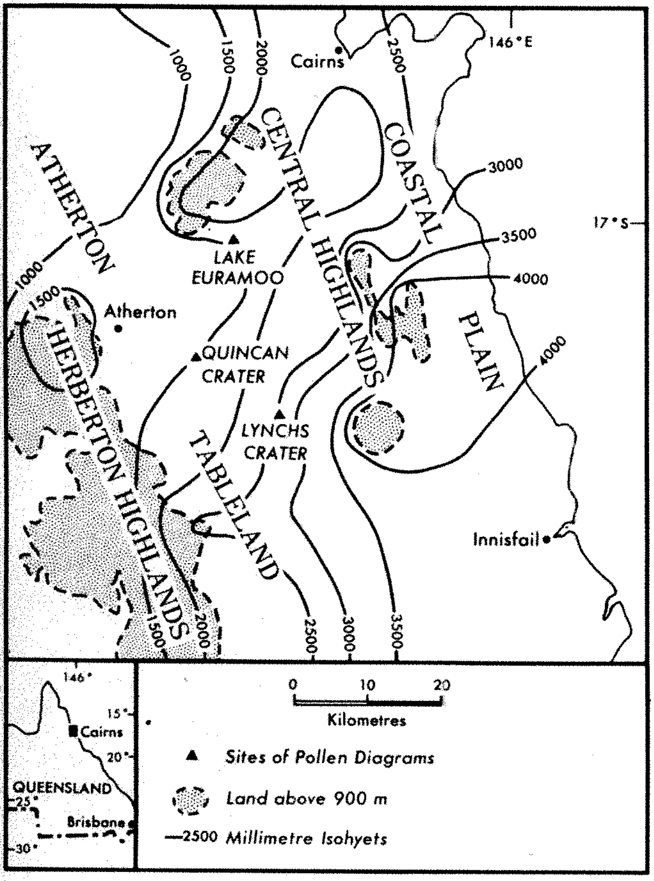

Fig. 1 Location of Lynch's Crater.

A core taken near the centre of the swamp provided 20 m of organic sediments for pollen analysis and radiocarbon dating. Selected results of pollen analyses at intervals along the core are shown in diagrammatic form on Fig. 2. Three distinct periods can be recognised. Zones F, E and D contain high percentages of the rain forest gymnosperms, *Podocarpus* and *Araucaria* and, except for zone E, low values of rain forest angiosperms; zone B is dominated by the sclerophyll taxa *Casuarina* and *Eucalyptus* comp.; zone A is mainly composed of rain forest angiosperm pollen. Zone C is transitional between earlier rain forest and later sclerophyll assemblages. The total number of dry land taxa recorded in each sample is highest in zones E and A and lowest in zone B.

Much of the pollen sequence is beyond radiocarbon dating range. It is difficult to extrapolate the age of the sediment to the base of the core because of variations in sediment type and probably in sedimentation rates, but it may lie between 60,000 and 70,000 years BP. It is suspected that the youngest date (6,850 ± 90 b.p. ANU-958), which corresponds with the zone A/B boundary, may not be accurate, since it could be contaminated by roots younger than the pollen analysed matrix.

Interpretation of the pollen diagram relies heavily on comparisons of the fossil spectra with spectra from surface samples collected within a variety of rain forest and sclerophyll vegetation types in north-east and south-east Queensland[4].

Pollen spectra of zone F correspond with moist araucarian forests (Low Microphyll Vine forests with *Araucaria*) which are extensive in southern Queensland but hardly present in the north-east of the state today. *Araucaria* is a common emergent above subtropical rain forest canopies on basaltic soils where the mean annual rainfall lies between 900 and 1,400 mm (L. J. Webb, communicated at Symposium on Ecological Research in Humid Tropics Vegetation, Kuching, 1965). It is therefore likely that rainfall was less than half the 2,500 mm received today during the period represented by zone F, though mean annual temperatures may have been little different from present day values. In zone E, higher percentages of Urticaceae/Moraceae and *Elaeocarpus* pollen at the expense of pollen from rain forest gymnosperm and sclerophyll taxa, as well as an increase in the total number of dry land taxa represented, indicate an increase in complexity of the surrounding vegetation. Complex Notophyll Vine forest with *Araucaria* probably replaced Low Microphyll Vine forest with *Araucaria* under increased precipitation (approximately 1,400 mm yr^{-1}). Zone D is very similar floristically to zone F and this suggests a return to Low Microphyll Vine forest under reduced rainfall.

The transition from Low Microphyll Vine forest to Sclerophyll vegetation occurred between about 38,000 and 27,000 years BP. The reason for this change could be a further decrease in precipitation but man, with his use of fire, may also have had an important effect, for it is now known that he has been in Australia for at least the past 32,000 yr (ref. 5). During the period of maximum sclerophyll expansion, rain forest patches which are not extensive today along the east coastal areas of Australia must have been very much restricted in area, and many rain forest types probably survived in stream gullies and other favourable retreats.

At some time before 7,000 BP, rain forest again invaded

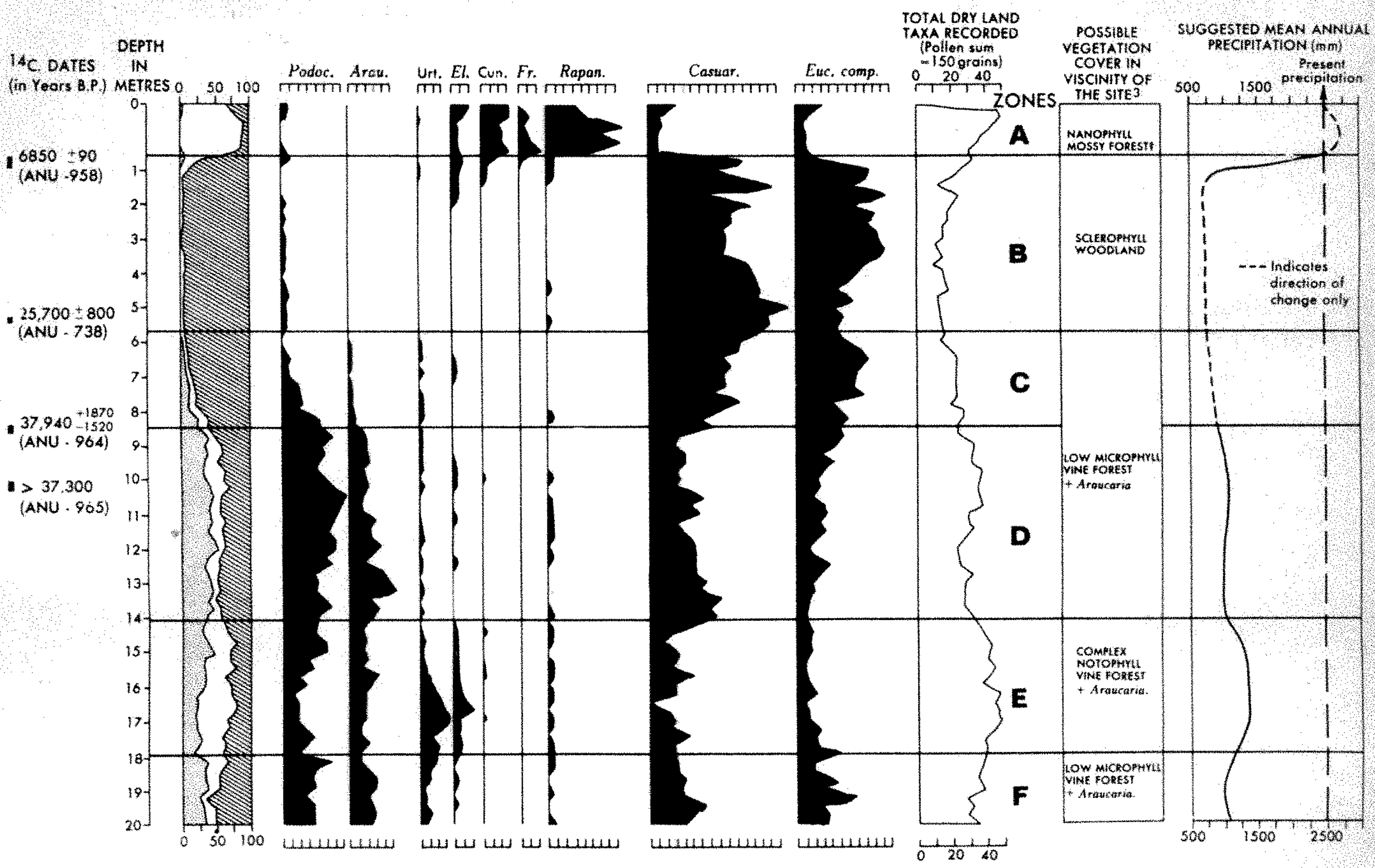

Fig. 2 Pollen diagram from Lynch's Crater. The column on the left shows the percentage of rain forest gymnosperms (stippled); rain forest angiosperms (white); sclerophyll taxa (hatched). The frequencies of pollen of all taxa are shown as percentages of the dry land plant pollen total; each division represents 5% of the pollen sum. Abbreviations for taxa: *Podoc.* = *Podocarpus; Arau.* = *Araucaria;* Urt. = Urticaceae/Moraceae: *El.* = *Elaeocarpus;* Cun. = Cunoniaceae; *Fr.* = *Freycinnettia; Rapan.* = *Rapanea; Casuar.* = *Casuariana; Euc. comp.* = *Eucalyptus comp.*

the area, certainly as a result of increased precipitation. High values of *Rapanea*, Cunoniaceae and *Freycinnettia* strongly suggest cooler and effectively wetter conditions than those pertaining today. The vegetation was probably a form of cool temperate rain forest (Nanophyll Mossy forest) and the absence of *Nothofagus* from this suggests that it had already become extinct in the region. The slight increase in sclerophyll pollen in the most recent spectrum possibly represents a decrease in effective precipitation towards the present day level. A more detailed interpretation of this zone is impossible at the moment as it is not known whether the pollen sequence is very condensed or whether the mud failed to accumulate through much of the period.

From the results presented, it is evident that climatic changes have occurred in north-east Australia of a magnitude similar to those of glaciated higher latitudes. The main variable, however, has been shown to be rainfall, with the latter part of the Pleistocene effectively drier than the Holocene. The effects of these climatic changes on the vegetation have been dramatic, three major vegetation types having been represented on the Atherton Tableland within the last 60,000 yr.

Work on Lynch's Crater is continuing; recent drilling has extended the recovery of core material beyond 40 m from the surface and still the deepest organic sediments have not been reached. When pollen from the total depth of material has eventually been analysed, the information recovered will provide a record of vegetation and climatic changes occurring through one or several complete glacial cycles and permit an informed assessment of the status of present-day vegetation patterns within the region.

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Experimental allergic neuritis induced by sensory nerve myelin may provide a model for nonlepromatous leprosy

Experimental allergic neuritis (EAN) is induced by the injection of peripheral nerve myelin and Freund's complete adjuvant into experimental animals[1]. It is characterised clinically by an acute motor paralysis accompanied by ataxia, both of which vary in severity from a mild to an extremely severe form. Histology shows focal areas of demyelination and mononuclear cell infiltration confined to the peripheral nervous system (PNS). It is one of a group of autoimmune delayed hypersensitivity conditions which are specific for target tissue but not for species. Experimental allergic encephalitis (EAE) is also an experimentally induced paralysing disease, but in this case the pathology is confined to the central nervous system (CNS). It is induced by injection of myelin of central nervous system origin in Freund's adjuvant. The observation that myelin from the CNS produced lesions confined to the CNS, whereas that from peripheral nerves produced lesions in the PNS has now been explained on the basis of biochemical differences between myelin from the two sites of origin[2,3].

Patients with nonlepromatous (tuberculoid or borderline) leprosy can develop acute sensory loss with selective involvement of superficial sensory modalities[4]. In such patients *Mycobacteria leprae* are few in number. In lepromatous leprosy, although bacteria are abundant, sensory loss occurs late in the disease, at a time when the bacteria have become scarce. This raises the possibility that the sensory loss may be an autoimmune reaction to target sensory nerve supplying the skin. If this is so it should be possible to demonstrate differences in antigenic properties between myelin from peripheral sensory (sural) nerve and that from mixed (sciatic) nerve, which we have previously used to produce EAN[5]. This might prove an experimental model for studying nonlepromatous leprosy. We report here some initial findings after observing for up to nine months, rabbits and rats which were injected with sural nerve in Freund's adjuvant. In all 9 rabbits and in 8 out of 16 rats, skin lesions suggestive of nonlepromatous leprosy were produced.

Human sural nerve obtained *post mortem* was used as antigen. Details of preparation are described elsewhere[5]. In nine Dutch Bantam rabbits 0.2 ml of the suspension was injected into the footpad of one hind limb and 0.05—0.1 ml injected similarly into 16 Sprague Dawley rats. Biopsies of skin lesions were taken from eight rabbits and four rats.

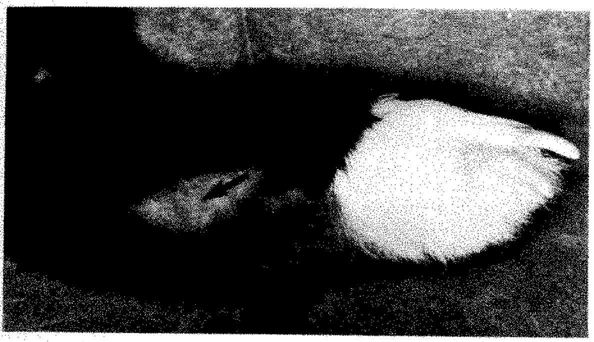

Fig. 1 Skin lesion of 3 week duration on dorsum of rabbit's foot 2 months after injection into same foot pad.

After 4 to 5 weeks the rabbits began to develop discrete skin lesions (Fig. 1) marked by loss of hair without skin ulceration. In some animals with coloured hair, there was a loss of pigment, which was sometimes incomplete, so that patches of hair were light orange, brown, or light grey. The initial lesions were mainly on the injected limbs but later they appeared on the face, other limbs, and dorsum of trunk. Most of the patches were transient, healing after several weeks, but a few have persisted for as long as 3 months. In some the hair began to grow again from the centre. The patches occurred in all nine rabbits, the total number of patches being 30. Some appeared as late as 6 months after injection. Similar denuded patches were observed in the rats. They occurred rather later and were seen in five out of six animals observed for 8 months, and in 10 rats under observations for the past 5 months three have developed patches. The total number was 12. Again some appeared as late as 7 months after injection. None appeared at the injection site. All the limb lesions occurred on the extensor surface, except two which overlapped slightly on to the flexor surface of the hind limb. Only one trunk patch was on the ventral surface. In six rabbits there was also analgesia to pinprick which was predominantly in the hind limbs and distal in distribution. In rats no convincing analgesia could be demonstrated.

Six of the nine rabbits also developed ataxia, with some motor loss, similar to that seen in EAN, when sciatic nerve was used as antigen source. Two of these animals died. The other four recovered from motor symptoms within 3 weeks. None of the 16 rats, including the eight with skin lesions, showed any ataxia or motor loss. Biopsy of the skin lesions showed erosion and disappearance of hair follicles in the early lesions. In a few there was also granuloma formation with mononuclear cells surrounded by lymphocytes. Dermal nerves showed a mononuclear cell infiltrate.

The clinical picture in the rabbits and rats using myelin from the purely sensory sural nerve as a source of antigen showed distinct differences from that seen when mixed (sciatic) nerve is used. The characteristic skin lesions which we have described have not been reported with sciatic nerve as antigen. Also the ataxia and motor loss which is such a prominent feature of the sciatic group did not occur in any of the rats although it did occur in six of the nine rabbits. In the sensory group the skin lesions appeared later than the classical EAN motor and ataxic signs and often ran a more prolonged course.

On this evidence different types of peripheral nerves may be assumed to differ from one another in antigenic potentiality. Biochemical analysis of the myelin from sural nerve has not been reported, but differences have been reported between sciatic nerve, dorsal roots and brachial plexus in the same species[6]; also the ultrastructural appearance varies slightly between fibres of different sizes in the CNS (ref. 7) and there are variations in the electrophoretic protein pattern of myelin from different parts of the CNS (ref. 8). EAN arising from injection of sciatic nerve has been suggested as a model for Guillain-Barré polyneuritis, a predominantly motor syndrome. The 'sensory' EAN which we have described resembles more closely the clinical and pathological features seen in nonlepromatous leprosy. The outstanding features of both are the discrete skin lesions characterised by lack of pigmentation, depilation, and healing from the centre, with a predeliction for the extensor surfaces[9]. Histologically our experimental skin lesions resemble those of early human leprosy[10], with erosion of hair follicles and mononuclear cell infiltration of dermal nerves. In a few of the experimental lesions there was also granuloma formation with mononuclear cells surrounded by lymphocytes.

Skin lesions similar to those in nonlepromatous leprosy have not previously been produced experimentally. The presence of lymphocytes in such skin lesions has been taken as evidence of a cell-mediated immune response directly to *M. leprae*[11]. But the injection of viable *M. leprae* into mice has failed to reproduce these skin lesions[12]. Our experiments suggest instead that *M. leprae* attack the Schwann cell or myelin of sensory peripheral nerves with release of antigen, the immune response of the patient being directed specifically against this antigen. The skin lesions of nonlepromatous leprosy are therefore essentially an autoimmune response to sensory cutaneous peripheral nerve.

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Functional and anatomical correlation of afferent responses from the isolated semicircular canal

THE semicircular canal is considered classically to be a sensor of angular head motion during animal navigation; however, the functional significance of the canal's morphology and innervation for transduction and encoding of specific head trajectories is unclear. Neural responses from single afferent fibres innervating the crista of isolated elasmobranch labyrinths were used by Groen *et al.* to determine linear system coefficients of an overdamped pendulum equation, considered to reflect directly the dynamics of the motion of the cupula–endolymphatic fluid in the ampulla[1]. Similar studies on other preparations revealed certain neural response characteristics that were at variance with simple pendulum models, and these differences were ascribed variously to differences in hair cell physiology and afferent or efferent innervation properties, whose effects were superimposed on the fundamental fluid pendulum dynamics[2–4]. But in addition to these hypotheses based on cellular influences, the topological organisation of the receptor could result in specific afferent response characteristics deriving from different anatomical regions within the crista. As evidence for the latter, we report diverse classes of afferent responses, describable by distinctly different linear system equations, that are correlated with the position of separate bundles innervating specific areas of the crista of the guitarfish, *Rhinobatos productus* (Fig. 1).

The horizontal ampullary nerve (HAN) separates from the utricular nerve as six to eight individual bundles which curve and proceed dorsally toward the ampulla (Fig. 1*a*). We counted myelinated fibres in the entire HAN of four preparations and found a mean of 1,359 (range 1,297–1,459) fibres. The individual nerve bundles varied in size, but the rostral-most and caudal-most generally contained fewer fibres (mean: 176, range: 87–367) relative to the central bundles (mean: 288, range: 201–577).

The individual nerve bundles, which appeared to retain their relative spatial distribution, converged near the ampulla, forming a nerve trunk which coursed along the major axis of the saddle-shaped crista (Fig. 6 in ref. 5) from its ventral to its dorsal extremities (Fig. 1*a*). In histological section planes through this major axis (receptor cells and nerves sectioned longitudinally), fibres nearest the crista separated and arched toward the hair cells lining the surface of the ventral planum semilunatum. The remaining fibres cascaded upward progressively along the ridge of the crista and finally projected into the dorsal planum semilunatum (Fig. 1*b*). Hence, fibres from the nerve trunk projected systematically to the crista along its ventral–dorsal axis.

A systematic projection of nerve fibres appeared also across the crista along a minor axis in the plane showing cross sections of both the nerve trunk and the crista (a rostral–caudal plane, Fig. 1*c*). Nerve fibres from the caudal portion of the nerve trunk were traced in serial sections to hair cells on the canal (caudal) slope, fibres from the central region to the crest of the ridge, and those from the rostral portion to the utricular (rostral) slope. This distribution of fibres was also apparent with a dissecting microscope. On the basis of microdissections and histological preparations, we conclude that a systematic pattern of innervation exists along both the major and minor axes of the horizontal crista.

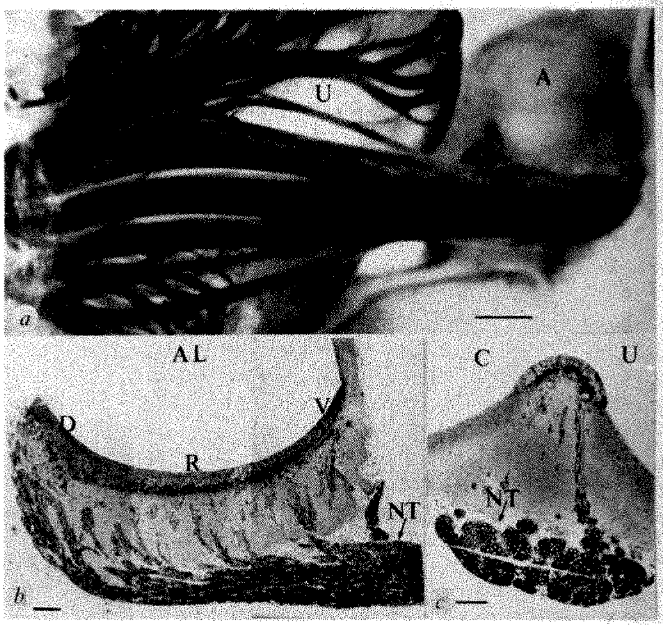

Fig. 1 *a*, Photomicrograph of the right horizontal ampullary nerve innervating the horizontal ampulla (A) of the guitarfish semicircular canal. Intact labyrinth specimens were stained with Sudan black B to facilitate microdissections. The ampullary nerves separate from those of the utricle (U) as individual bundles (numbered in a rostral–caudal sequence) which project dorsally to converge into a nerve trunk (NT) as they approach the ampulla. The nerve trunk courses along the long axis of the crista. The saddle-shaped horizontal crista projects into the ampulla in a medial-lateral plane at nearly right angles to the plane of this figure. The marker equals 0.2 mm. *b*, A longitudinal section through the centre of the left ampullary lumen (AL) and the sensory region, the crista (V,R,D). Labyrinths for histological preparation were fixed *in situ* with glutaraldehyde-paraformaldehyde in 0.1 M phosphate buffer containing 10% sucrose after cervical dislocation and rapid removal of the surrounding cartilage. After embedding in Araldite 502, the tissue was sectioned at 0.5 μm and stained with aqueous toluidine blue. The plane of this figure is rotated 90° about the long axis of the crista relative to (*a*). Fibres from the nerve trunk (NT) closest to the crista separate and cascade systematically toward the receptor cells, first to the ventral planum semilunatum (V), then along the ridge (R), and finally to the dorsal planum semilunatum (D). The marker equals 0.1 mm. *c*, A cross section of the nerve trunk (NT) and the crista. The plane of this section is perpendicular to the minor axis of the crista and oriented 90° relative to the plane of (*b*). The nerve fibres project from the anterior portion of the nerve trunk toward receptor cells on the slope facing the utricular opening of the semicircular canal (that is, rostrally). The marker equals 0.1 mm.

We recorded single-unit spike trains by suspending severed nerve sub-bundles in air with a forceps electrode[1]. Spike trains were amplified, transmitted by means of frequency modulation telemetry, recorded and displayed conventionally. The preparation was aligned on the rotational axis of a Goerz/Inland rotating table (model 800) with the horizontal semicircular canal plane oriented parallel to that of the table.

We developed a new technique to characterise sensory systems on-line by cross-correlating their afferent response to a white noise pseudorandom binary acceleration stimulus (PRBS)[6-8]. Cross-correlations of the spike train responses with six to sixteen periods of the PRBS stimulus were computed on-line and scaled appropriately to obtain estimates of the linear system unit impulse response (UIR) for each afferent[9]. The PRBS input afforded significant advantages over the use of conventional deterministic inputs (for example, sinusoidal and constant acceleration): accurate small-signal linear characteristics were determined over wide bandwidths in a fraction of the time required for conventional techniques, and other noise sources that were uncorrelated with the input did not corrupt the cross-correlated results[7,10]. The accuracy of the UIR as an estimator of the afferent input–output characteristics was tested by observing whether responses to other inputs could be predicted correctly by linear convolution[9,10]. Conventional inputs were applied experimentally in addition to the PRBS input, and the percentage mean-square-error (% MSE) of the predicted and experimental outputs was computed. Most units showed a sufficiently small % MSE (<15%) to justify using linear estimation for this system.

Our analysis is based on results from 120 cells. We found a pattern of organisation in the UIR characteristics that was correlated with relative position of the parent bundles (Fig. 2). Responses from central bundles, for example, 3 and 4 in Fig. 1*a*, had the form shown in Fig. 2a–c; an initial deflection followed by a decay toward the baseline with a slight overshoot indicative of adaption[2]. In contrast, the responses from the rostral and caudal bundles, for example, 1 and 6 in Fig. 1*a*, had the contours shown in Fig. 2d–f: a resolvable rising component followed by a slower decay toward the baseline analogous to an overdamped second-order linear system impulse response[11]. Responses from bundles 2 and 5 were mixed and included all contours shown in Fig. 2. Variability was found in both the relative magnitudes of the initial points and the time constants of the rise and decay components as determined by a nonlinear regression program. The peak amplitudes of all responses from bundles 1 and 6 were similar to those in Fig. 2d–f, indicating lower sensitivities in these afferents relative to those from central bundles. We conclude that the type of response was indeed correlated with relative location of the parent bundles which project to specific anatomical regions of the crista.

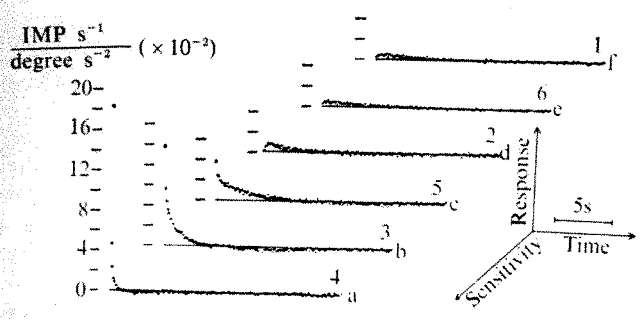

Fig. 2 Linear system impulse response estimates obtained by cross-correlating pseudorandom binary white noise inputs with spike train outputs from six afferents innervating the left horizontal semicircular canal of the guitarfish. Isolated labyrinths were prepared for afferent response recording by pithing the brain, removing the cranium and cartilage at the rear of the orbit to expose the horizontal ampullary nerve, and mounting the labyrinth on a rotating table. A pseudorandom binary sequence (PRBS) with white noise properties, generated by a PDP-11 computer program, was converted to a sequence of rotational accelerations of magnitude ± 100 degree s^{-2} and direction that varied randomly within each PRBS period. The stimulus pattern was repeated periodically with a period $N\Delta t$ as determined by the duration Δt of individual PRBS states and the total number N of states in each period. The PRBS stimulus has a spectral density that is theoretically constant (within 3 dB), and is by definition a white noise source over the band from $1/N\Delta t$ to $1/3\Delta t$ Hz, approximately. We used values of $\Delta t = 92$ ms and $N = 255$ to result in a period of 23.5 s and a noise bandwidth from 0.04 to 4 Hz. Correction factors were applied to compensate for input transducer error resulting from the rotating table's finite switching time and the PRBS finite bandwidth. The adjacent points are separated by 92 ms intervals. The relative location of the parent bundle of each afferent is indicated in the figure by number for each response beginning at the most rostral bundle in the nerve fan. Responses (a)–(c) are typical of those from bundles located centrally in the nerve fan (numbered 3 and 4) and exhibit an initial peak of varying magnitude (for example, 18.3 in (a) followed by an approximately exponential decay and overshoot of the baseline. Responses (d)–(f) are typical of those from bundles located at the rostral and caudal extremities of the nerve fan and exhibit a resolvable exponential rise followed by an exponential decay with negligible overshoot of the baseline analogous to an overdamped second-order linear system. Sensitivity is defined arbitrarily as the magnitude of the response predicted (by linear convolution) to a step input of magnitude 1 degree s^{-2} and duration 0.46 s, that is, the sum of the amplitudes of the first five points in each impulse response.

We excluded the possibility that the response diversity derived from time-dependent degenerative changes in the isolated preparation. Long term recordings (3–8 h) from cells showing responses typical of all types shown in Fig. 2 never showed changes in afferent response characteristics of individual cells during these periods. Furthermore, in paired cell recordings obtained simultaneously using separate electrode channels, typical rostral–caudal and central bundle responses were recorded simultaneously, both immediately and several hours after exposure of the nerve.

These results showing a functional and anatomical correlation of semicircular canal afferent responses suggest an alternative to the view that the afferents encode direct responses to mechanically simple fluid-cupula displacements. Specifically, more complex transductive mechanisms could result in local filtering of the receptor's physiological inputs for the purpose of enhanced signal detection. As an analogy, ideal receivers for known signals corrupted by noise, such as occur in radar, use bandpass filters matched to a particular signal in the sense that the latter is detected in the presence of Gaussian noise with a theoretically optimum signal-to-noise ratio[12]. The filter's impulse response is a time-reversal of the matched signal's waveform. The afferent fibres of the semicircular canal could thus be considered an ensemble of bandpass filters that are matched, in a signal detection theory sense, to specific head acceleration trajectories determined by each afferent's impulse response reversed in time. Each afferent could then be considered tuned to a particular range of head accelerations analogous to the afferent encoding of specific stimulus features described for other sensory systems[13].

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Dissociation of lymphocyte activating determinants from recognition sites in mouse MLR

PREVIOUSLY, we were able to block mixed lymphocyte reactions (MLR) in man using anti-light chain antibody[1] but we have been unable to confirm this observation in subsequent studies using other anti-light chain sera We have now found that MLR in the mouse is unaffected by a variety of anti-immunoglobulin sera but using alloantisera it has become evident that the stimulator cells, but not the responder lymphocytes, can be blocked

Female mice of 5–6 weeks of age of the following inbred and F_1 hybrid strains were used BALB/c, C57BL, CBA/H, C3H, BALB/c×CBA/H F_1, BALB/c×C57BL F_1, BALB/c×DBA/2 F_1, CBA/H×AKR/J F_1 and BALB/c×C3H F_1 (breeders obtained from the Laboratory Animals Centre, Carshalton, Surrey) Rabbit antisera to mouse immunoglobulins were prepared as described elsewhere[2] BALB/c anti-C57BL and C57BL anti-BALB/c alloantisera were raised by one skin grafting followed by six weekly injections of C57BL and BALB/c spleen cells respectively into male and female recipient mice One spleen was used for six mice C57BL anti-BALB/c serum and BALB/c anti-C57BL serum showed 90% cytotoxicity to a titre of 1/100 and 1/80 respectively Normal BALB/c and C57BL sera as well as all antisera were stored at −40° C

Foetal calf serum (FCS, Difco, Detroit, Michigan), normal sera and mouse antisera were inactivated for 30 min at 56° C The normal mouse sera and alloantisera were absorbed for 2 h in a water bath at 28° C, with CBA/H (Tables 1, 2) or syngeneic (Table 3) spleen cells (1 spleen per ml of serum) to eliminate nonspecific inhibitory factor(s) The spleen cells were washed twice with Eagle's medium (MEM, Wellcome, Beckenham, England), before they were used for absorption

Lymph node suspensions were prepared as previously described[3,4] except that the cells were finally resuspended in equal ratios of RPMI 1640 (Gibco, Grand Island, New York), and Eagle's medium with 5% FCS

Parent and F_1 lymphocytes at the concentrations of $2\ 66\times 10^6$ ml^{-1} were mixed with 5% absorbed normal serum or appropriate antiserum and then mixed in equal volumes to give a final concentration of $2\ 5\times 10^6$ cells ml^{-1} These parent-F_1 MLCs (refs 1 and 5) were set up in 0 5 ml volumes in each E2R culture tube between selected combinations, incompatible for H–2 or M–locus The sera were allowed to remain in the

Table 1 Specific blocking of stimulating cells in MLC with C57BL anti-BALB/c

Responding cells (H–2, Mls)	Stimulating cells (H–2, Mls)†	^{14}C–Thymidine incorporation* on day 3 5 when cultured in presence of			
		Normal C57BL serum	Index of stimulation	C57BL anti-BALB/c serum	Index of stimulation
C57BL (b,b)	—	823± 70		852± 88	
—	(C57BL×BALB/c)F_1 (bd,bb)	63± 16		75± 21	
C57BI	(C57BL×BALB/c)F_1	4,991± 73	11 3	1,008±154	2 2
—	(C57BL×CBA/H)F_1 (bk,bb)	1,104± 118		322± 49	
C57BL	(C57BL×CBA/H)F_1	7,221± 101	9 6	5,195±134	8 8
CBA/H (k b)	—	900± 30		842± 43	
—	(CBA/H×AKR/J)F_1 (kk,ba)	986± 70		833± 38	
CBA/H	(CBA/H×AKR/J)F_1	24,206±1,265	25 7	21,633±590	25 8

Normal C57BL and C57BL anti-BALB/c sera were decomplemented, absorbed with CBA/H spleen cells to remove nonspecific inhibitory factor and used at the final dilution of 5% Experiments performed using C57BL anti-BALB/c serum, decomplemented and absorbed with syngeneic (C57BL) spleen cells showed that the blocking effect is specific to the stimulating cells and absorption of alloantiserum with syngeneic or third party spleen cells makes no difference'

* Expressed as mean c p m ml^{-1} of triplicates ± s e † Mls = M–locus

Table 2 No effect of C57BL anti-BALB/c alloantiserum on responding cells in MLC

Responding cells (H–2,Mls)	Stimulating cells (H–2,Mls)†	^{14}C-Thymidine incorporation* on day 3 5 when cultured in presence of			
		Normal C57BL serum	Index of stimulation	C57BL anti-BALB/c serum	Index of stimulation
BALB/c (d,b)	—	755± 52		757± 63	
—	(BALB/c×C57BL)F_1 (db,bb)	869± 71		281± 13	
BALB/c	(BALB/c×C57BL)F_1	15,385±788	18 9	13,677±770	26 3
—	(BALB/c×CBA/H)F_1 (dk,bb)	4,385±126		2,740±270	
BALB/c	(BALB/c×CBA/H)F_1	11,951±801	4 6	13,356±740	4 9
—	(BALB/c×DBA/2)F_1 (dd,ba)	3,405± 21		2,465±148	
BALB/c	(BALP/c×DBA/2)F_1	14,823±430	7 1	12,216±436	7 6
CBA/H (k,b)	—	4,316±130		3,107±118	
—	(CBA×AKR/J)F_1 (kk ba)	4,390± 92		3,554±216	
CBA/H	(CBA/H×AKR/J)F_1	43,276±371	9 9	39,327±933	11 8

* Expressed as c p m ml^{-1} of triplicates ± s e † Mls = M–locus

Table 3 No effect of BALB/c anti-C57BL alloantiserum on responding cells in MLC with specific blocking of stimulating cells

Responding cells (H–2,Mls)	Stimulating cells (H–2,Mls)†	^{14}C–Thymidine incorporation* on day 3 5 when cultured in presence of Normal BALB/c serum	Index of stimulation	BALB/c anti-C57BL serum	Index of stimulation
BALB/c (d,b)	—	434± 28		364± 23	
—	BALB/c×C57BL(F$_1$ (db,bb)	425± 32		489± 27	
BALB/c	(BALB/c×C57BL)F$_1$	2,226×132	5 3	824± 45	1 9
—	(BALB/c×CBA/H)F$_1$ (dk,bb)	354± 8		381± 26	
BALB/c	(BALB/c×CBA/H)F$_1$	2,750±176	7 0	2,536±120	6 8
—	(BALB/c×DBA/2)F$_1$ (dd,ba)	2,553± 67		1,792± 73	
BALB/c	(BALB/c×DBA/2)F$_1$	4,096±142	2 7	10,357±497	9 6
C57BL (b,b)	—	597± 57		1,133± 61	
C57BL	(BALB/c×C57BL)F$_1$	4,641±376	9 1	8,144±300	10 0

Normal BALB/c and BALB/c anti-C57BL sera were decomplemented, absorbed with syngeneic BALB/c spleen cells to remove nonspecific inhibitory factor and used at the final dilution of 5%

* Expressed as c p m ml^{-1} of triplicates±s e † Mls = M–locus

cultures throughout the experiment The cultures were refed with equal ratios of Eagle's and RPMI with 5% FCS on day 2 ^{14}C–Thymidine incorporation was measured on day 3 5 according to the technique previously described[4,5] Index of stimulation was calculated as counts in parent plus F$_1$ MLC divided by mean value of parent and F$_1$ cultured alone

Rabbit antisera to immunoglobulin light chains, Fab fragment or the various heavy chain subclasses in no instance inhibited MLR between H–2 incompatible cells, if anything, they were at times responsible for some stimulation (Table 4)

Table 1 shows the results of experiments in which the anti H–2 alloantisera directed against the F$_1$ stimulator cells are introduced into H–2 complex incompatible parent-F$_1$ mixed cultures C57BL lymph node cells were responding to BALB/c×C57BL F$_1$ target lymph node cells The antiserum was absorbed with third party (CBA/H) or syngeneic (C57BL) spleen cells Both methods of absorption were equally effective in removing nonspecific inhibitory activity of mouse serum The controls were of two kinds—the third party F$_1$ cell was from BALB/c×CBA/H F$_1$ lymph nodes and the third party MLC involved an unrelated H–2 compatible, M–locus incompatible combination, that is, CBA/H (H–2^k, Mls^b) and CBA/H×AKR/J F$_1$ target (H–2^k/H–2^k, Mls^b/Mls^a) The results show that only when the alloantiserum was directed towards the specificity of stimulating cells was there 'blocking' of MLR (the stimulation was reduced from 11 3 to 2 2 times the background) There was no significant reduction of stimulation with any of the controls Experiments were then done in which the antiserum was specifically directed against the responding cell in MLC Results of representative experiments are shown in Tables 2 and 3 When anti-H–2^d serum (C57BL anti-BALB/c) was directed against the BALB/c (H–2^d) responding cells in MLC, no blocking was obtained with any of the H–2 or M–locus incompatible combinations (Table 2) Similarly, when another antiserum was used (BALB/c anti-C57BL), blocking of MLC was observed only when C57BL antigens were present on the stimulating rather than the responding cells

Our studies therefore do not provide any evidence for the involvement of immunoglobulin determinants (as recognised in the rabbit antisera used) in the recognition of allogeneic cells in mouse MLR Similar negative findings were obtained in attempts to inhibit T-cell cytotoxicity in the P815 mastocytoma system using the same anti-immunoglobulin reagents (W V, I M R, and D B Thomas, unpublished) in agreement with other authors[6] We have shown, however, that H–2 alloantisera directed against the stimulating cell, but not the responding cell, can specifically block MLR We cannot be certain which determinants the serum is reacting against since it is likely to contain antibodies against both the H–2K and H–2D specificity as well as the lymphocyte activating determinants Our first experiments were with sera which had been absorbed with third party cells to remove nonspecific inhibitory factors and while this was unlikely to affect blocking of the stimulating cells, it is possible that antibodies to receptor sites, if present, might have been removed by this procedure This objection was met by showing that selective blocking of stimulating cells was still obtained after absorption of the nonspecific inhibitor with syngeneic cells

If antibodies had in fact been raised against the MLR recognition units then they were not of sufficient titre, avidity or specificity to block the reaction Another interpretation could be that the antisera did in fact react with the receptors of the responding cells but that no inhibition occurred because the blocked receptors were then rapidly shed and new ones synthesised What these results do show is that the activating determinants are distinct from the receptor sites In this sense, the results are in conflict with those of Ceppellini[7] or Thorsby and Solheim[8] in man but are consistent with other findings (J Brochier, I M R, and H F, unpublished) A reason for this discrepancy might lie in the concentration of alloantiserum used by the former authors, if the lymphocyte activating

Table 4 Lack of effect of rabbit anti-mouse immunoglobulin sera on the mouse mixed lymphocyte reaction

Experiment	1				2				3		
Parent+F$_1$ combination (H–2,Mls)	CBA+CBA×BALB/c (k b)+(kd,bb)				CBA+CBA×BALB/c				BALB/c+BALB/c×C3H (d,b) (dk,bc)		
Antiserum added	NRS	Anti LC(1)	Anti LC(2)	Anti IgM	NRS	Anti Fab(1)	Anti Fab(2)	Anti IgM	NRS	Anti (IgG1+IgA)	Anti (IgG2a+b)
Lymphocytes											
Control	510	857	940	530	322	172	170	228	708	882	1,000
Parent+F$_1$	3,541	6,816	11,439	2,912	1,404	503	769	1,019	1,426	3,312	4,099
Index of stimulation	6 9	7 9	12 2	5 5	4 4	2 9	4 5	4 5	2 0	3 8	4 1

Figures represent ^{14}C–thymidine incorporation in c p m (mean of triplicates) on day 3 5 Control values were calculated as half the sum of counts in parental and F$_1$ cells cultured alone Index of stimulation calculated as counts in parent+F$_1$ cultures/counts in control NRS, Normal rabbit serum, LC, light chain determinant and the recognition site are distinct but, nonetheless, present on the same molecule or molecular complex, one could envisage the possibility that higher antibody levels might be required to block the responding cell than to mask the determinant on the stimulating cell

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High frequency of T lineage lymphocytes in nude mouse spleen

CONGENITALLY athymic mice (nude mice) lack the T cell compartment of the lymphoid system[1-3] and their spleen has been extensively used as a source of B cells It has been shown recently, however, that nude mice have a few T cells[4] and precursors for T cells[5] These precursors can repopulate a grafted neonatal thymus and differentiate further[5], migrate to secondary lymphoid organs[5] and restore some T-cell-dependent immunity[6]

Although the T precursors probably originate primarily in the bone marrow, the nude mouse spleen might also contain some precursors Indeed, a variety of treatments renders within a few hours a proportion of nude mouse spleen cells, presumably lymphocytes, susceptible to cytotoxic anti-T lymphocyte antisera[7] Moreover, it is claimed that cultivation of nude mouse spleen cells on thymic reticuloendothelial cell monolayers also induces an important rise in characteristic T cell reactivities[8]

When analysed by immunofluorescence, as many as 40% of nude mouse spleen lymphocytes lack detectable membrane immunoglobulin (Ig), (ref 5 and F L and G E R, unpublished) a B cell marker[9] When reacted with fluorescent mouse antibody directed against the θ antigen[9,10], a T lymphocyte marker[9], up to 20% of nude mouse spleen lymphocytes show a faint fluorescence which is specific (cells are stained with anti-θ–C3H reagents not with anti-θ–AKR) but too weak to be satisfactory In preliminary experiments the detection of θ was amplified by using in sequence anti-θ antibody and rhodamine-labelled anti-mouse Ig, a procedure which also stains the B cells through their surface Ig Calculating the difference between the number of stained cells whether the first step was anti-θ or normal mouse serum, again about 20% of nude mouse spleen lymphocytes seemed definitely to have the θ marker

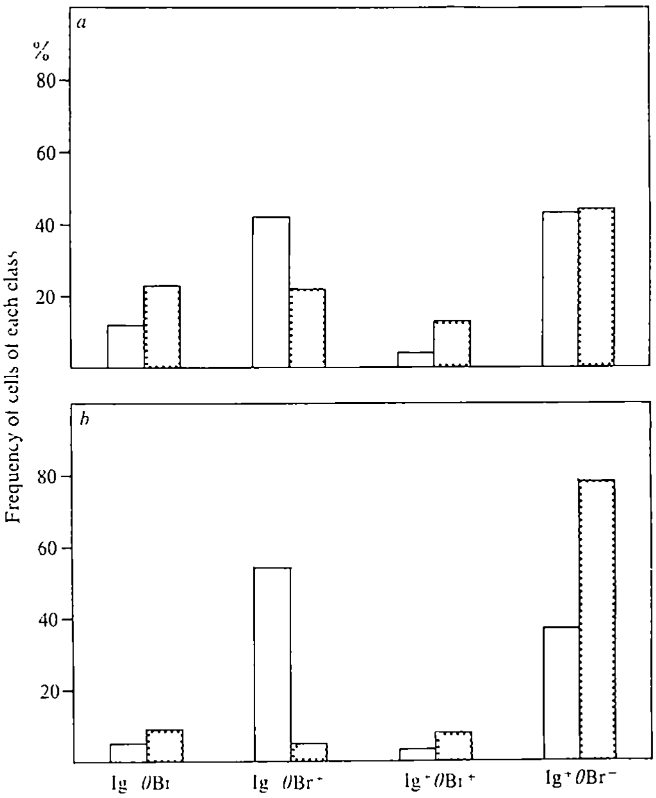

Fig 1 Frequency of cells with or without membrane Ig and/or θBr 2.5×10^6 cells in 0 1 ml medium (RPMI 1640+0 5% BSA+10 mM NaN_3 buffered with 0 03 M HEPES and NaOH to *p*H 7 2) were incubated (30 min, 0° C) with 0 1 ml (50 μg) of RIgG–α–MθBr (NRIgG as control), washed three times, and further incubated (15 min, 0° C) with 0 2 ml of a mixture of TRITC–SIgG–α–RIg (50 μg) and FITC–SIgG–α–MIg (50 μg), washed, and examined for double fluorescence The figure represents mean values of the frequencies of cells from seven normal mice (five BALB/c, two NMRI) and seven nudes (five BALB/c-nu, two NRMI-nu) (at least 500 cells were examined per individual mouse) *a*, Spleen lymphocytes, *b*, lymph node lymphocytes Open columns, normal mice, stippled columns, nude mice For the preparation of RIgG–α–MθBr, immunisation was by two intramuscular injections of 3 CBA/J mouse brain, homogenised in complete Freund's adjuvant at 1 week intervals boosted 5 months later before a wait of 2 weeks and the collection and pooling of immune serum A pool of preimmune serum from 10 rabbits was used as a control The isolation of IgG was by repeated $(NH_4)_2SO_4$ precipitation (1 6 M, 0° C) followed by DEAE-cellulose chromatography (*p*H 6 3, 0 0175 M phosphate buffer) The *in vivo* absorption of the non–α–θ antibody in nude mice BALB/c–nu, fourth backcross Gl Bomholtgard Ltd, Ry, Denmark) was by intraperitoneal injections of 10 mg IgG ml^{-1} per nude mouse, bled out 8–10 h later The isolation of the RIgG from the mouse serum was by the same procedure as above Most of the mouse Ig (90–95%) sticks to the DEAE, the RIgG passes through The IgG was concentrated on Diaflo membrane UM2 up to 10–20 mg ml^{-1} Storage was at 4° C, in PBS+0 5% BSA+10 mM NaN_3 We used RIgG–α–MθBr on cell suspensions at a final concentration of 250 μg ml^{-1} As a control we used NRIgG prepared from the preimmune serum pool and absorbed similarly

To use the amplification provided by indirect fluorescence and at the same time discriminate between B ($Ig^+\theta^-$) and T ($Ig^-\theta^+$) lymphocytes within a single cell preparation we used the anti-θ antibody present in antisera raised in rabbits against the mouse brain associated θ antigen (θBr)[11] after *in vivo* absorption in nude mice (see legend to Fig 1)

The basic technique for the detection of membrane markers on viable cells by immunofluorescent reagents in conditions which do not allow polar redistribution have been described in detail[5,10,12,13] Binding of RIgG–α–MθBr was revealed in indirect immunofluorescence using sheep anti-rabbit Ig antibody conjugated with fluoresceine or tetramethylrhodamine (FITC- and TRITC–SIgG–α–RIg)[12] Mouse membrane Ig was detected with FITC- or TRITC–SIgG–α–MIg[12]

When assayed on thymus, spleen and lymph node cells from normal BALB/c and AKR mice (either sex, 8–12 weeks old, Gl Bomholtgard Ltd, Denmark), the RIgG–α–MθBr stained the expected percentages of T lymphocytes[9] No significant binding of NRIgG was detected The specificity of binding for cells of the T lineage was assessed by performing a double labelling of θ and θBr AKR or BALB/c mouse cells were first stained by TRITC–C3H IgG–α–θAKR or TRITC–AKR IgG–α–θC3H respectively[5,10], then reacted with RIgG–α–MθBr (NRIgG as control) which was further detected by FITC–SIgG–α–RIg The expected proportion of T lymphocytes was labelled in spleen and lymph node cell preparations[9] and those cells were labelled with both reagents Moreover, preincubation of BALB/c or AKR lymph node or spleen cells with AKR–α–θC3H or C3H–α–θAKR alloantiserum (diluted 1 40, 30 min, 0° C) virtually blocked further binding of the RIgG–α–MθBr Normal AKR and C3H sera had no inhibitory activity Thus, the rabbit heteroantibody, although not detectably discriminating between θC3H and θAKR, seems to bind to the same or very close membrane determinant(s) than the mouse alloantibody whose specificity was fully assessed earlier[5,10]

The binding characteristics of RIgG–α–MθBr are similar to those of α–θ alloantibody both bind better to thymus lymphocytes than to peripheral T cells, both give a homogeneous binding appearing as a 'ring' fluorescence when detected by fluorescent monovalent Fab–α–Ig Spots or caps are induced only if a second layer of divalent α–Ig antibody is added

Double staining for Ig and θBr was performed Lymphocytes could be classified into four categories typical B cells having only membrane Ig (Ig$^+$θBr$^-$), typical T cells having only the θ antigen (Ig$^-$θBr$^+$), cells lacking detectable amounts of both markers (Ig$^-$θBr$^-$) and cells expressing both markers (Ig$^+$θBr$^+$) Normal and nude mice (BALB/c, NMRI, BALB/c–nu and NMRI–nu, Gl Bomholtgard, Ry, Denmark) were compared (Fig 1)

In nude mouse spleen the proportion of typical B cells (Ig$^+$θBr$^-$ lymphocytes) is not higher than in normal mouse spleen Interestingly, about one fifth of the lymphocytes are definitely of T lineage (Ig$^-$θBr$^+$) In lymph nodes typical B cells are more frequent in nudes than in normal mice and many fewer lymphocytes are of T lineage In both spleen and lymph nodes the frequency of Ig$^-$θBr$^-$ and Ig$^+$θBr$^+$ is higher in nudes than in normal mice

When spleen cells from a pool of BALB/c–nu were preincubated with AKR–α–θC3H alloantiserum (diluted 1 40 for 30 min at 0° C) there was an 84% reduction in the number of cells binding detectable amounts of RIgG–α–MθBr (compared with preincubation with normal AKR serum) This shows that, as in normal mice, in nude mice RIgG–α–MθBr also binds apparently to the θ antigen itself and not to an undefined 'stem cell antigen'[14,15]

NRIgG did not detectably bind to the cells, except in the case of one NMRI and one NMRI–nu where rare cells picked up NRIgG unspecifically, most of them having also Ig on their surface These false double positive cells accounted for a quarter of those found when the RIgG–α–MθBr was used on the cells from the same animal

Ig$^+$θBr$^-$ cells in both normal and nude mice seem to be typical B lymphocytes and do not require further comment but it is interesting that nude mice have a high frequency of lymphocytes of T lineage The 20% of Ig$^-$θBr$^+$ lymphocytes in nude spleen are not equivalent to those of normal mice, indeed the latter can also be easily detected as θ$^+$ by the mouse α–θ alloantibody used in direct immunofluorescence, while the definite detection of the former depends on the large amplification produced by the indirect immunofluorescence method used These cells have less θ antigen and it is not known if they are still potential precursors or cells of T lineage having followed an aberrant differentiation pathway because of the lack of thymus

Ig$^-$θBr$^-$ cells which might be 'early precursors' have already been described[16,17], but their possible T nature was rejected[17] on the assumption that the only cells of T lineage homing in the spleen have to come from the thymus

The significance of Ig$^+$θBr$^+$ cells is difficult to evaluate as it is not known if both markers are the actual synthetic products of the cells

Cells binding the heavily iodinated synthetic polypeptide (Tyr,Glu)–Ala– –Lys (ref 18) were found among all four cell types indicating that commitment to antigen and receptor expression on cells of T lineage do not require the thymic presence

The various cell types described here, and the possibility of a prethymic differentiation pathway of T cells, clearly need further characterisation It is quite clear, however, that nude mice are not a source of pure B cells, especially when the spleen is used Conclusions on lymphocyte activation based on this assumption might have to be reconsidered

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Restriction by H-2 gene complex of transfer of cell-mediated immunity to *Listeria monocytogenes*

RESULTS from three distinct experimental systems indicate that, except for lymphocytes reacting to alloantigens, interaction between sensitised thymus-derived lymphocytes (T cells) and other somatic cells occurs only in syngeneic or semiallogeneic systems First, histoincompatible T cells do not function as helpers for antibody-forming cell precursors (B cells)[1,2] Second, maximal proliferation of lymphocytes (presumably T cells) resulting from exposure *in vitro* of sensitised guinea pig lymphocytes to antigen-pulse macrophages is observed only when the two cell types share major histocompatibility antigens[3] Third, cell-mediated lysis of fibroblasts or macrophages infected with lymphocytic choriomeningitis (LCM) virus, a function of specifically sensitised T cells, occurs only when lymphocyte and target cells share at least one set of H-2 antigenic specificities[4,5] The phenomenon is unaffected by differences either in minor histocompatibility antigens, or at the M locus

Can this restriction be applied more generally as an index of T cell involvement in immunological phenomena? This possibility can be further investigated in another model where T cells are known to play a central part, adoptive transfer of immunity to *Listeria monocytogenes* infection in mice[6-8] Experiments reported here indicate that T cell activity in this experimental system is also restricted by the H-2 gene complex

The strain of *Listeria monocytogenes*, and methods used for immunisation, cell preparation, cell transfer and enumeration of viable bacteria in individual organs have been described elsewhere[7,9,10] In most experiments $5 0\times10^7$ CBA/H×C57BL F_1 6 d *Listeria* immune or normal cells were injected intravenously (i v) into CBA/H or BALB/c recipients, infected i v 2 h previously with 5×10^3 viable *Listeria* Protective effects were measured quantitatively by determining viable bacterial counts in individual recipient spleens and livers at intervals after cell transfer

Growth kinetics of *Listeria* in CBA/H and BALB/c mice were comparable (controls, Fig 1) Immune CBA×C57BL F_1 cells protected semiallogeneic parental CBA/H much more effectively than allogeneic BALB/c cells (Fig 1) Viable bacterial counts in spleen were a more sensitive indicator of protection than liver counts Therefore although liver counts were also determined, only viable counts in spleen will be presented, the results for liver being essentially similar Transfer of normal cells into infected semiallogeneic or allogeneic recipients had no significant influence on bacterial counts in spleen or liver during the first 2 d In the following experiments recipient mice were killed 42 to 48 h after cell transfer[7,8,10]

The degree of protection of CBA/H or BALB/c mice by adoptively transferred immune CBA/H×C57BL F_1

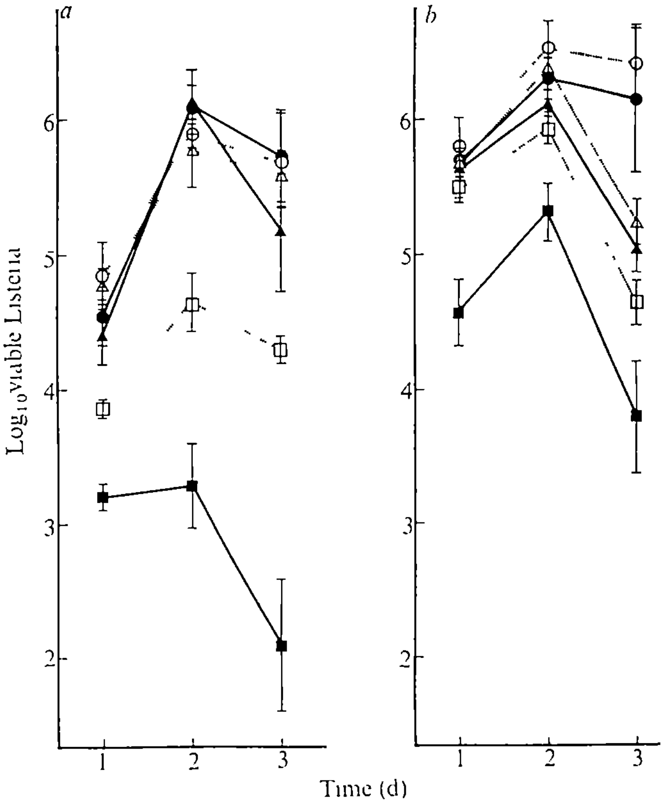

Fig 1 Effect of the transfer of 5×10^7 immune (■, □) or normal (▲, △) spleen cells (CBA/H × C57BL F_1) as compared with controls (●, ○) on the viable counts of *Listeria monocytogenes* in *a*, spleens and *b*, livers of parental strain CBA/H (■, ▲, ●) or allogeneic BALB/c (□, △, ○) recipients infected with 5×10^3 viable bacteria 2 h before cell transfer Each point represents the mean of groups of four to five mice The vertical bars enclose two standard errors of the mean Control mice and normal cell recipient did not differ significantly, except liver counts on day 3 CBA/H $P < 0 05$, BALB/c $P < 0 01$ Protection by immune cells was significant when compared with no cells or normal cells in CBA/H spleens day 1 $P < 0 01$, day 2 $P < 0 001$, day 3 $P < 0 01$, livers day 1 $P < 0 02$, day 2 $P < 0 02$, day 3 $P < 0 05$ For BALB/c spleens day 1 $P < 0 01$ day 2 $P < 0 02$, day 3 $P < 0 02$, livers day 1 $P < 0 05$, day 2 $P < 0 05$, day 3 $P < 0 05$ Difference of protection in CBA/H and BALB/c spleen day 1 $P < 0 01$, day 2 $P < 0 01$ day 3 $P < 0 001$, liver, day 1 $P < 0 05$, day 2 $P < 0 05$, day 3 $P < 0 05$

Table 1 Protective effect of transferred immune cells on syngeneic, semiallogenic, and allogenic recipients

Donors (H-2 type)	Recipients (H-2 type)	Log_{10} viable bacteria counts immune cells*	 normal cells†	Protection
Experiment 1				
CBA/H × C57BL F_1 (k/b)	CBA/H × C57BL F_1 (k/b)	3 12 ± 0 21§	5 71 ± 0 15	2 59
CBA/H × C57BL F_1 (k/b)	CBA/H (k)	3 31 ± 0 27§	6 10 ± 0 19	2 80
CBA/H × C57BL F_1 (k/b)	C57BL (b)	1 97 ± 0 15§	5 03 ± 0 10	3 06
CBA/H × C57BL F_1 (k/b)	BALB/c (d)	4 64 ± 0 20+	5 45 ± 0 20	0 81
Experiment 2				
CBA/H (k)	CBA/H (k)	3 43 ± 0 15§	5 28 ± 0 07	1 85
CBA/H (k)	CBA/H × C57BL (k/b)	3 43 ± 0 11§	5 49 ± 0 11	2 06
CBA/H (k)	BALB/c (d)	4 72 ± 0 15‡	5 30 ± 0 06	0 58¶
CBA/H (k)	C57BL × BALB/c F_1 (b/d)	4 39 ± 0 18§	5 28 ± 0 07	0 89¶
BALB/c (d)	BALB/c (d)	3 63 ± 0 19§	5 49 ± 0 11	1 86
BALB/c (d)	CBA/H (k)	4 40 ± 0 09‡	5 32 ± 0 13	0 92¶
Experiment 3				
DBA/2 (d)	DBA/2 (d)	4 64 ± 0 23§	6 10 ± 0 09	1 46
DBA/2 (d)	BALB/c (d)	4 37 ± 0 21‡	5 99 ± 0 15	1 62

* Mean ± s e m of groups of five mice significance determined with Student's *t* test against normal cells

+ $P < 0 05$

‡ $P < 0 01$

§ $P < 0 001$

† Controls (no cells), experiment 1 CBA/H 6 10 ± 0 12, BALB/c 5 89 ± 0 11, F_1 6 13 ± 0 15, experiment 2 CBA/H 5 39 ± 0 19, BALB/c 5 68 ± 0 17, F_1 5 50 ± 0 18

¶ Significance of protection in syngeneic or semi-allogeneic against allogeneic recipients $P < 0 01$

5×10^7 viable normal or immune spleen cells were transferred to recipients Immune cells were from donors injected with 2–3 $\times10^3$ viable bacteria 6 d previously Recipients were infected with 5×10^3 viable bacteria 2 h before cell transfer Log_{10} viable bacteria were counted in spleens of recipients 42 h after cell transfer spleen cells and the restriction of their efficiency to the semiallogeneic donor–recipient combination are clearly dose dependent (Fig 2) Parental CBA/H mice were protected by 1 7×10^7 or more immune F₁ cells (P<0 001), but not by 6×10^6 cells BALB/c recipients were only protected by 5×10^7 (P<0 01) or more allogeneic immune cells, but to a lesser extent than CBA/H A very high cell dose, 1 5×10^8 immune cells, was obviously not much more efficient than 5×10^7 cells in the semiallogeneic host whereas BALB/c were better protected The two dose-response relationships, plotted on a log/log basis, were linear and parallel over certain dose ranges (Fig 2) The efficiency with which the protection is transferred into semiallogeneic CBA/H and allogeneic BALB/c could therefore be compared and differs by a factor of about 4

Under the chosen conditions 5×10^7 transferred immune cells caused comparable protection in syngeneic or semiallogeneic (F₁ into parent or parent into F₁) hosts and revealed marked restriction of the protective effects in allogeneic recipients (Table 1) As the viable counts in the spleens of control (no cells) mice and of normal cell recipients did not differ significantly in the various strains (except for C57BL), the protective effect of the immune cells could be readily compared

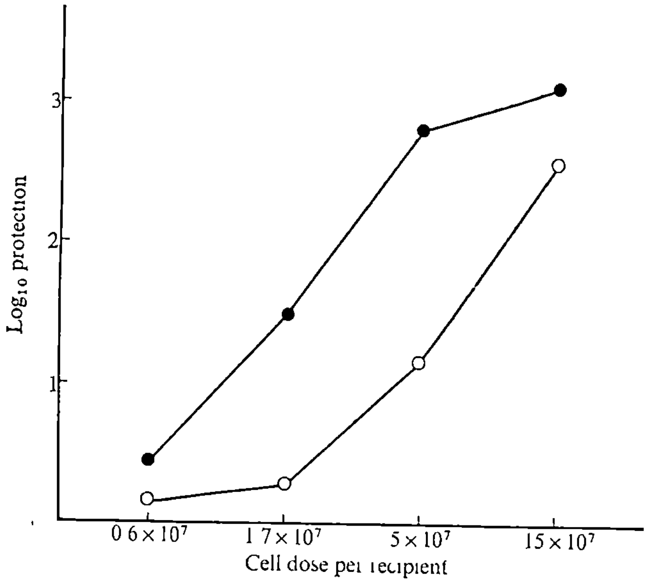

Fig 2 Protection against *Listeria monocytogenes* by the transfer of various doses of immune spleen cells (CBA/H × C57BL F_1) as compared, with normal cells, into parental strain CBA/H (●) or allogeneic BALB/c (○) receipients, determined as the difference of the mean of $\log_{10}$ viable counts in the spleens of groups of four to five mice Significance of difference 0 6 × 10^7 cells n s , 1 7 × 10^7 cells P < 0 02, 5 × 10^7 cells P < 0 01, 1 5 × 10^8 cells n s

The possibility that a difference at the M locus is responsible for this restriction was investigated by transferring DBA/2 *Listeria*-immune spleen cells into DBA/2 and BALB/c recipients Both strains share H-2^d antigenic specificities[11], but differ at the M locus (Table 1, experiment 3) Incompatibility at the M locus had no obvious effect

How can the restriction be explained? As only 40 to 48 h elapse between cell transfer and determination of viable bacterial counts, graft *versus* host (GvH) reactions are unlikely to influence adoptively transferred cells Also the donor recipient combination CBA/H×C57BL F₁ into CBA/H or BALB/c should give comparable conditions for GvH reaction and homing of transferred cells in both recipient strains Furthermore, cytotoxic T cells from mice infected with LCM virus home in comparable numbers to the spleens of LCM infected syngeneic or allogeneic recipients on day 1 after cell transfer (unpublished data)

Events leading to protection against *L monocytogenes* in the recipients of transferred immune cells can be summarised as follows *Listeria* organisms injected i v are taken up by fixed recipient macrophages[9] *Listeria* antigens would thus be present in lesions either as free bacterial antigens or associated with living or dead macrophages Sensitised donor lymphocytes injected later are presumably triggered on contact with the antigen and then probably produce soluble factor(s)[12,13], which activate macrophages to the increased bactericidal activity responsible for protection[9] Donor macrophages play no significant role in protection[6–8]

There are potentially two points in this sequence of events at which restriction by the H-2 gene complex could operate

First, it is possible that the activation of macrophages by a soluble factor only occurs efficiently when macrophages and factor are from the same mouse strain There is no strict precedent for such a phenomenon, but the species specificity of interferon[14,15] and mouse migration inhibition factor (MIF)[16] could be a similar phenomenon This possibility could be readily tested in systems where macrophage activation is achieved *in vitro*[12,13]

Second, as antigens presented on the surface of macrophages trigger T cells more efficiently than soluble antigen[3,4], the alternative possibility is that antigen presented by histoincompatible macrophages fails to trigger T cells efficiently[3] Both possibilities are compatible with the observation that immune allogeneic cells can protect a host if sufficiently large doses of cells are transferred

Similar mechanisms have been proposed in other systems For antigen-induced dose-dependent proliferation of primed lymphocytes by antigen-pulsed macrophages to occur, both cell populations must share histocompatibility antigens[3] When soluble antigen is added in excess, the restriction by histocompatibility antigens is overcome[3] In contrast a secondary *in vitro* anti-DNP response of mouse spleen cells could be triggered equally well by syngeneic and allogeneic macrophages pulsed with DNP-conjugate[17] This could be explained by excess T helper activity being present in the system, thus covering up differences in the efficiency of antigen presentation by histocompatible or incompatible macrophages[3,18]

In conclusion, these results further favour the idea that mouse T cell functions in general may be restricted by the H-2 gene complex

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Reactivity of steroid-resistant neonatal thymocytes

THE reactivity of thymus-derived (T) lymphocytes when stimulated with the phytomitogens phytohaemagglutinin (PHA), concanavalin A (con A) and pokeweed mitogen (PWM), correlates well with immunological reactivity Consequently, the mitogens have been useful in studies of the functional maturity of thymocytes in foetal and neonatal mice Reactivity to con A and PWM increases greatly during the perinatal period, but PHA responsiveness reaches a peak shortly before birth and then declines[3] This decline could be due to either (1) reversible suppression of PHA-responsive thymocytes by PHA-unresponsive cells, or (2) the emigration or death of PHA-reactive cells or the immigration or expansion of a population of PHA-unresponsive cells If PHA-responsive thymocytes in the newborn mouse are all steroid resistant, as seems to be the case in the adult[4-6], then steroid treatment of newborns might aid the choice between possibilities (1) and (2) If (1) is correct, steroid treatment should cause a greater increment in thymocyte PHA responsiveness than could be explained by the increased relative number of highly reactive steroid-resistant cells If (2) is correct, steroid treatment should increase PHA-responsiveness in proportion to the increase in relative number of PHA-responsive cells If, however, all PHA-responsive cells are eliminated, steroid treatment should not increase PHA responsiveness This possibility is given some support by a report[5] involving techniques different from ours We have therefore attempted to characterise the fraction of thymocytes surviving steroid treatment in the newborn mouse with regard to mitogen reactivity, reactivity in the mixed lymphocyte reaction (MLR), and surface representation of the θ alloantigen We found that steroid treatment increased the PHA responsiveness of newborn thymocytes, supporting explanation (2)

BALB/c mice about 24 h old were given 100 mg kg^{-1} hydrocortisone acetate intraperitoneally 48 h before death, together with colloidal carbon to mark parathymic lymph nodes, which were discarded Controls were given only colloidal carbon Thymocytes were cultured at 2 × 10^{6} ml^{-1} in microtitre trays for 72 h in RPMI 1640 supplemented with 5% foetal calf serum, 2 mM glutamine, 15 mM HEPES buffer, and 50 μg ml^{-1} gentamicin PHA-P was used at a final concentration of 1 μg ml^{-1}, con A was used at 2 μg ml^{-1}, and PWM at 3 μg ml^{-1} Allogeneic target cells were prepared by incubating C57BL/6 spleen cells for 30 min at 37° C in 40 μg ml^{-1} mitomycin C, followed by three washes in Hanks balanced salt solution (BSS) One microcurie of ^{3}H-thymidine (specific activity 6 7 Ci mmol^{-1}) was added to each culture well 16 h before termination Cultures were collected and ^{3}H-thymidine incorporation into trichloroacetic acid-precipitable material was measured in a scintillation counter

Steroid-treated newborn and adult mice yielded about 16% of the number of thymocytes obtained from untreated controls Table 1 shows a comparison of the mitogen responses of newborn and adult normal and steroid-resistant thymocytes Steroid-resistant newborn thymocytes had substantially more PHA reactivity than normal newborn thymocytes This was true if data were expressed as c p m or as the ratio of experimental to control The increment in reactivity, however, was no more than would be anticipated if steroid-resistant thymocytes were the only PHA-reactive cells in the newborn thymus, that is, the steroid-resistant fraction, constituting about one-sixth of all thymus cells, responded six times as well as unfractionated thymocytes This suggests that diminished PHA reactivity in the neonate is not due to strong reversible suppression by steroid-sensitive thymocytes of the PHA-reactive subpopulation, nor to the absence of PHA-reactive cells due to emigration or cell death

Reactivity of newborn steroid-resistant thymocytes to con A and PWM was substantially greater than that of normal newborn thymocytes As in the case of PHA, the steroid-resistant subpopulation could account for all the con A and PWM-reactive newborn thymocytes

In the adult, steroid-resistant thymocytes had much greater PHA, con A and PWM-reactivity than normal thymocytes As in the newborn, con A and PWM-reactivity in the adult thymus could be accounted for by the steroid-resistant fraction, the steroid-resistant thymocytes were even more reactive to PHA than would be expected These experiments show that there are comparable numbers of mitogen-reactive cells in the newborn and adult thymus after steroid treatment If mitogen responsiveness is used as a marker of T cell maturation, then the newborn thymus, like the adult, contains a small pool of functionally mature T cells

In both newborns and adults, steroid treatment resulted in only a small increment in reactivity to allogenic (C67BL/6) spleen cells, confirming earlier reports[6] This may reflect a paucity of MLR-reactive cells in the thymus, or it may be due to a requirement for more than one effector cell type in the MLR

The ability of thymocytes surviving steroid treatment to absorb the cytotoxic activity of AKR anti-θ C3H serum was used as an index of the relative amount of θ alloantigen expressed on the surface of the cell population A constant number of normal or steroid-resistant thymocytes was incubated with a 1 4 dilution of high titre anti-θ serum for 30 min at 4° C Cells were pelleted by centrifugation, and the residual titre of the supernatant (compared with a control tube containing no cells) assayed against ^{51}Cr-labelled adult BALB/c thymocytes as before[7] Absorption with adult normal thymocytes markedly reduced the cytotoxic titre (Fig 1) of the anti-θ serum, whereas adult steroid-resistant cells caused only a small reduction This confirms reports[8]

Table 1 Mitogen responses of newborn and adult steroid-resistant thymocytes

	No mitogen	PHA	Con A	PWM
Newborn thymocytes	1,099 ± 239	2,203 ± 181	5,509 ± 671	5,885 ± 593
Newborn steroid-resistant thymocytes*	963 ± 157	17,406 ±1,763	35,256 ±6,535	28,265 ±3,336
Adult thymocytes	263 ± 38	1,336 ± 92	18,837 ±1,353	4,201 ±1,067
Adult steroid-resistant thymocytes*	581 ± 285	27,200 ±2,296	28,389 ±3,994	21,792 ±2,204

Numbers represent the means of c p m ± s e of replicates of four per group in one representative experiment Cells were cultured for 72 h with 3H-thymidine added for the final 16 h of culture

* Newborn or adult BALB/c mice received 100 mg kg^{-1} hydrocortisone intraperitoneally 48 h before removal of thymuses for culture

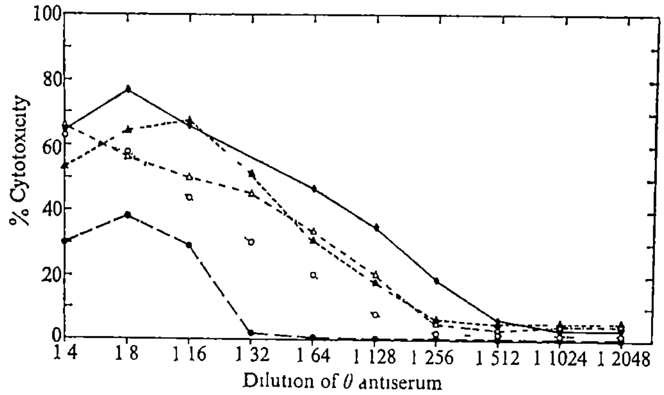

Fig 1 Cytotoxicity curve for anti-θ C3H serum absorbed with different thymocyte populations and then titred against ^{51}Cr-labelled adult thymocytes ⊙, Newborn thymocytes, △, newborn steroid-resistant thymocytes, ●, adult thymocytes, ▲, adult steroid-resistant thymocytes, ◆, unabsorbed anti-θ (C3H)

that normal thymocytes express many θ determinants per cell, while the steroid-resistant subpopulation expresses on the average many fewer θ determinants per cell

In contrast, however, newborn BALB/c thymocytes (mice <24 h old) from normal mice reduced the antiserum titre much less than comparable numbers of adult thymocytes Moreover, steroid treatment caused only a marginal reduction in the ability to absorb anti-θ activity We conclude that newborn thymocytes express somewhat fewer θ determinants per cell than do adult thymocytes, and that, consequently, steroid treatment leads to a smaller shift in the relative θ expression of the surviving subpopulation

Steroid treatment had similar effects on the morphology of newborn and adult thymus, almost obliterating the thymic cortex In newborns, however, we noted a few collections of small lymphocytes in the subcapsular region

We conclude that the potential decline in PHA responsiveness is not due to the selective disappearance from the thymus of PHA-responsive steroid-resistant lymphocytes On the contrary, all the PHA reactivity of the neonatal thymus can be accounted for by the steroid-resistant cells It thus seems unlikely that there are many PHA-reactive cells in the steroid-sensitive fraction comprising 80%–90% of thymocytes Our studies also suggest that the perinatal decline is not due to suppression of PHA-reactive cells by steroid-sensitive thymocytes, for the abolition of such suppression by steroid treatment should have led to a greater increase in PHA reactivity than we observed The decline in PHA responsiveness, then, could be due to dilution of PHA-reactive cells by PHA-unresponsive cells, or to the emigration of PHA-reactive cells from the thymus We cannot distinguish between these possibilities although significant numbers of PHA-reactive cells do not appear in the neonatal spleen until 6 or 7 d after the sudden decline in thymic PHA-responsiveness, arguing against emigration to the spleen Emigration to lymph nodes is a possibility that has not been investigated

The finding that poorly-reactive normal neonatal thymocytes and highly-reactive steroid-resistant neonatal thymocytes appeared to express relatively similar amounts of surface θ alloantigen suggests that in this instance θ does not serve as a marker for cell differentiation, as proposed for the adult thymus[9]

Finally, these data emphasise the relative maturity, at least in terms of mitogen reactivity, of cells in the thymus of newborn mice

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Dissociation of EEG and behavioural effects of ethanol provide evidence for a noncholinergic basis of intoxication

IN 1957 it was reported that physotigmine, a drug that potentiates brain acetylcholine by inhibiting the enzyme which normally destroys it, could partially antagonise a tranquilliser, such as chlorpomazine[1] Many years elapsed before anyone tested physostigmine for possible antagonistic effects against other sedative type drugs, such as ethanol Physostigmine given before but not after massive doses of ethanol (4 5 g kg^{-1}) did seem to shorten sleeping time and to reduce death of mice, an observation that led to the conclusion that ethanol might inhibit acetylcholine release or function[2,3]

I thought that this question ought to be examined using more specific measures of ethanol action, in particular the electroencephalogram (EEG) and a battery of behavioural tests for intoxication Such studies revealed that physostigmine blocked ethanol-induced EEG deactivation (changes to high voltage, slower frequencies) but did not prevent behavioural signs of intoxication This to my knowledge is a rare demonstration of dissociation of the EEG and behavioural effects of ethanol, although similar dissociations have been reported for reserpine[4], immobility reflex ("animal hypnosis")[5,6], human hypnosis[7,8], paradoxical or dream stage of sleep[9], and certain comatose disease states of dogs[10–12] and humans[13] The most significant aspect of the ethanol dissociation is that it suggests that intoxication does not primarily involve impairment of cholinergic systems

EEG recording was performed on eight rats, with chronically implanted epidural electrodes bilaterally placed over frontal, parietal, and occipital cortex During recording, rats were free to move within a 25 cm^2 box

Four standard 'catalepsy' tests were used in a separate study of behaviour In one test, a 'cork test', the rat was placed on four corks of diameter 3½ cm, one foot on each, with corks spread 8 cm apart laterally and 10 cm apart longitudinally, catalepsy was scored if feet were not moved for 10 s Two 'platform' tests were used in which both front paws were placed on an elevated platform, either 3 cm high or 9 cm high, catalepsy was defined as holding that position for 10 s Finally, a 'net' test was used in which the rat was placed on a vertically positioned hardware cloth (5 mm square), 25 cm above the floor, catalepsy was defined as staying on the net for 15 s

All drugs were administered intraperitoneally in the following doses physostigmine salicylate (eserine), 0 2 mg kg^{-1} (a 'teeth chattering' dose), neostigmine methylsulphate (0 2 and 0 3 mg kg^{-1}) (the latter caused collapse and was near the fatal limit), and ethanol, 20% v/v aqueous solution, 1 5 and 2 g kg^{-1} (both were intoxicating, with the latter causing the most pronounced EEG signs)

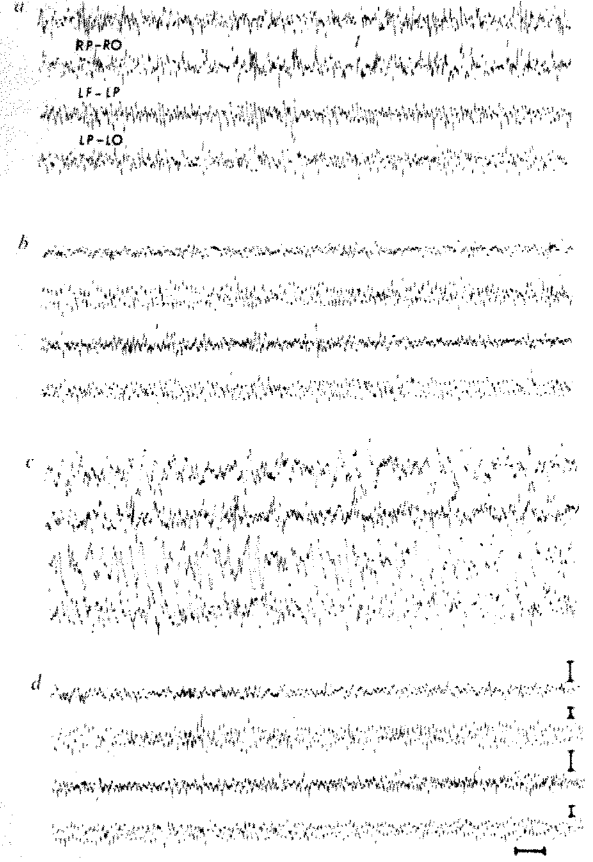

Fig. 1 Representative illustrations of the EEG phenomena exhibited by all rats. All records are from the same freely behaving rat, with 3–4 d between successive trials. *a*, Control records indicate a mildly activated EEG. *b*, Eserine (2 mg kg^{-1}) caused a marked activation (note 6–7 s^{-1} activity) which began 4 min after injection and lasted the full 15 min recording session. *c*, Alcohol (2 mg kg^{-1}) caused pronounced deactivation, beginning 3 min after injection and persisting throughout the recording period. *d*, Administration of eserine 5 min before alcohol injection blocked alcohol-induced deactivation, with EEG activation quite evident for the full 15 min. Behavioural tests of this rat and the others during this eserine + alcohol activation clearly revealed that the rats were behaviourally intoxicated (see Fig. 2). RF, LF = right and left frontal leads: RP, LP = right and left parietal; RO, LO = right and left occipital. Calibrations: 100 μV, 1 s.

Signs of EEG-behavioural dissociation were observed transiently when ethanol alone was given. In all but one rat, both 1.5 g kg^{-1} and 2 g kg^{-1} induced behavioural intoxication during EEG activation that began within 2 to 6 min after injection and lasted at least 1.5 to 10 min for 1.5 g kg^{-1} and 1.5 to 5 min for 2 g kg^{-1}. There are a few reports of ethanol-induced excitatory effects in terms of EEG[14–17] and in terms of enhanced evoked responses[18–21]. Behavioural intoxication during such periods has not been reported, but my rats showed typical subjective signs of intoxication (mild ataxia, hypokinesis, bumping into objects, falling off the edge of a table, flaccid muscle tone and toppling over). During the EEG activation two rats were disconnected from the EEG machine and immediately given the catalepsy tests, which verified their behavioural intoxication.

The EEG-behavioural dissociation was still more conspicuous and readily demonstrated in rats given both physostigmine and ethanol. Physostigmine alone produced persistent EEG activation, beginning within 3 to 8 min and lasting at least 15 min (Fig. 1). When given 5 min before 2 g kg^{-1} ethanol, the EEG activation persisted in every rat for the full 15 min recording period, whereas this dose of ethanol always produced sustained EEG deactivation within 3 to 7 min (Fig. 1). Nonetheless, physostigmine-pretreated rats were clearly intoxicated. Physostigmine not only prevented the EEG deactivating property of ethanol, but to a lesser extent reversed it. Giving physostigmine 5 min after 2 g kg^{-1} of ethanol, at which time the EEG was usually deactivated, caused within several minutes EEG activations which alternated with deactivated periods in some rats. Again, rats displayed subjective signs of intoxication while their EEG was activated.

While it is likely that lower doses of physostigmine would have been less effective in blocking the EEG effect of ethanol the fact that a large dose did block shows there was an antagonism which was restricted to the EEG. A higher dose of physostigmine might in theory have also antagonised the intoxicated behaviour, but such a dose is toxic; in fact, signs of toxicity (shivering, teeth chattering) were universally present at the 0.2 mg kg^{-1} dose. The important point is that there is a dose of physostigmine at which EEG and behavioural effects of ethanol are clearly dissociable.

Physostigmine effects on the EEG were shown to be central, because neostigmine (0.2 and 0.3 mg kg^{-1}), which has the same action but does not cross the blood–brain barrier, failed to produce persistent EEG activation when given alone and of course also failed to prevent ethanol-induced deactivation.

In the battery of catalepsy tests, untreated rats did not show signs of catalepsy, but rats given 2 mg kg^{-1} ethanol failed to move from either the low or high platform (Fig. 2). The unexpected negative results on the cork test seemed due to compulsive slithering movements; rats moved immediately off of the corks and then resumed their hypokinesis. The results of the net test, though quantitatively the same as with saline, were qualitatively quite different. Sixty per cent of saline-injected rats stayed on the net more than 5 s before climbing down; ethanol-injected rats had so little muscle tone that they could not hold on at all.

Eserine, at the dosage used, caused mild teeth chattering, shivering, and hypokinesis (accounting for positive platform tests). The positive results of the net test reflected the skeletal muscle spasms which promoted clutching of the wire by all feet, making removal of the feet difficult.

When eserine and ethanol were given in combination, irrespective of which was given first, the rats displayed all the superficial signs of intoxication, such as ataxia, hypokinesis, and diminished postural tone. These signs were coexistent with the overt signs of eserine effects of shivering and teeth chattering. The failure of eserine to antag-

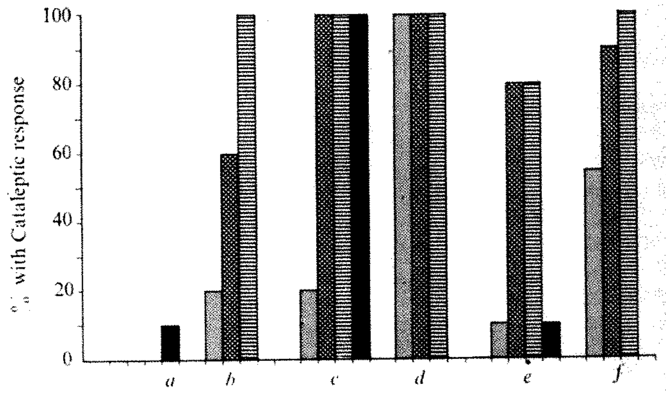

Fig. 2 Behavioural effects of *a*, saline; *b*, alcohol; *c*, eserine; *d*, alcohol and eserine; *e*, neostigmine; *f*, alcohol and neostigmine. Data from the 10 rats per group are expressed as the percentage which shows a cataleptic response, compared to the pretreatment performance of each rat. Vertical bars show the response after each treatment to (from left to right) cork test; 3 cm platform test; 9 cm platform test; net test.

onise the behavioural intoxication was clearly documented in the net test, where all rats fell off the net, because of hypotonia. A surprising and unexplained positive cork test was noted in all rats given the combined treatment.

Neostigmine alone caused rats to fail the net test; rats fell off from apparent weakness, suggesting that much of the body tension caused by eserine was not arising at the neuromuscular junction. As expected, neostigmine when combined with ethanol also caused failure on the net test. There may have been a significant failure ($P<0.05$) on the cork test as well; neostigmine–ethanol treated rats seemed to have a compulsive squirming tendency but were too weak to move, whereas rats treated with eserine and ethanol made no attempts to move.

This work reveals that ethanol caused motor deficits more or less independently of cortical influences. Investigators may have been tempted to ignore subcortical and cerebellar sites as targets of ethanol action because ethanol has been reported to deactivate the EEG in animals with rostral brain stem transections[22]. A noncortical locus is also suggested from studies showing a similar dissociation of EEG and behavioural effects caused by stimulants such as amphetamine[23,24]; such drugs prevented ethanol-induced deactivation of the EEG, but did not prevent behavioural signs of inebriation.

The present work also points to a noncholinergic basis of intoxication. A similar conclusion can be drawn from the recent report that pharmacological manipulation of the cholinergic system did not alter ethanol effects on a discriminated lever-press avoidance task[25].

Such demonstrations of a noncholinergic basis for behavioural intoxication are not really in conflict with a recent report that acetylcholine release is suppressed by ethanol[26]. Such inhibition would account for ethanol-induced EEG deactivation, because acetylcholine mediates neocortical activation (reviewed in ref. 27). But the cortical action of ethanol could be secondary to general decreased neuronal activity, and in any case, it would not necessarily cause behavioural intoxication. Likewise, the motor deficits would not have to be mediated cholinergically. Some recent support for a noncholinergic basis for behavioural intoxication comes from the recent report that physostigmine and atropine did not affect ethanol withdrawal reactions in addicted mice, whereas such reactions were altered by pharmacological manipulation of brain catecholamines and γ-aminobutyric acid[28].

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Ultrastructural evidence for foetal liver injury induced by *in utero* exposure to small doses of methylmercury

THE ability of organomercury compounds, particularly methylmercury, to concentrate in tissues of fish and other animals at levels which are toxic for human consumption, is of increasing environmental concern[1–3]. The propensity for mercury compounds to induce morphological, biochemical and enzymatic alterations in the kidneys and the central nervous system is well known[4–8]. Because the liver has been shown to accumulate a large fraction of mercury after administration of methylmercury, it should also be considered a target organ of methylmercury toxicity[9]. Hepatic injury and changes in several hepatic enzyme systems have been reported[10].

Several investigators have shown that methylmercury compounds, when administered to pregnant mice, rats and monkeys rapidly pass through the placenta in the developing foetal tissues[11,12]. Recently, ultrastructural evidence was reported for foetal nephropathy after *in utero* exposure to methylmercury (our unpublished results). We report here, however, the first ultrastructural demonstration of foetal liver injury induced by maternal exposure early in gestation to small doses of methylmercury.

Twenty-four pregnant female Sprague-Dawley rats (average body weight 250 g) were divided into four groups of six animals each, individually housed and permitted free access to water and Purina lab chow. Methylmercuric chloride (CH_3HgCl) was injected subcutaneously into groups 1, 2 and 3 in respective doses of 0.5, 0.75 and 1.0 mg on the ninth day of their gestational cycle. Group 4 control animals similarly received an equal volume of sterile physiological saline.

Neurological symptoms were not observed in pregnant female rats treated with methylmercury and no gross teratological malformations were noted in any of their offspring. Newborn pups were anaesthetised immediately with ether and their livers were excised surgically. Slices of liver were diced into small cubes and immersion fixed in mixture of 2% glutaraldehyde and 0.5% paraformaldehyde in 0.1 M phosphate buffer, *p*H 7.3, for 4 h. They were then rinsed in phosphate buffer, post-fixed in buffered 2% osmium tetroxide, dehydrated in graded ethanol and propylene oxide and embedded in Epon. Thin sections were

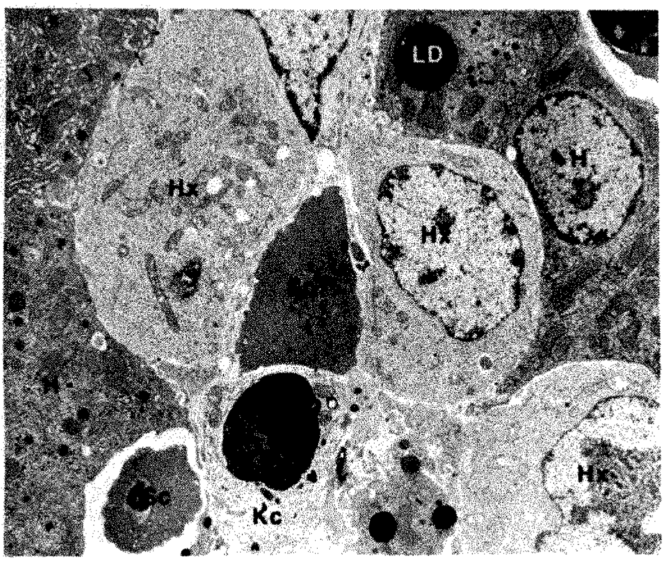

Fig. 1 Foetal hepatic parenchyma after maternal exposure to 1.0 mg CH_3HgCl (3.0 p.p.m. mercury) on the ninth day of gestation. Several hepatocytes (Hx), which are being extruded into the sinusoidal spaces, show an absence of general cellular organelles except for degenerating mitochondria and free ribosomes. Prominent dilation of endoplasmic reticulum (arrows) as well as sinusoidal casts (Sc) of cellular material were also observed. Küpffer cells (Kc) contain giant lysosomes (L). (× 1,860).

counter-stained with uranyl acetate and lead citrate for electron microscopy. Thin coronal sections of the liver were fixed in buffered 10% formalin, dehydrated in ethanol and embedded in paraffin. Sections (5 μm) were stained with haematoxylin and eosin, and by the periodic acid-Schiff technique for light microscopic examination.

Light and electron microscopic evaluation of the foetal liver of animals treated *in utero* with sterile physiological saline revealed no significant morphological alterations. In contrast, foetuses from mothers exposed to methylmercury during gestation presented a consistent pattern of liver injury, the severity and extent of which were dose dependent. The most severe pathological alterations were observed in foetal livers exposed *in utero* to 1.0 mg of CH_3HgCl. At the light microscopic level these changes were confined primarily to hepatocytes and consisted of vacuolation, an increase in lysosomal profiles and in many cases, cellular necrosis.

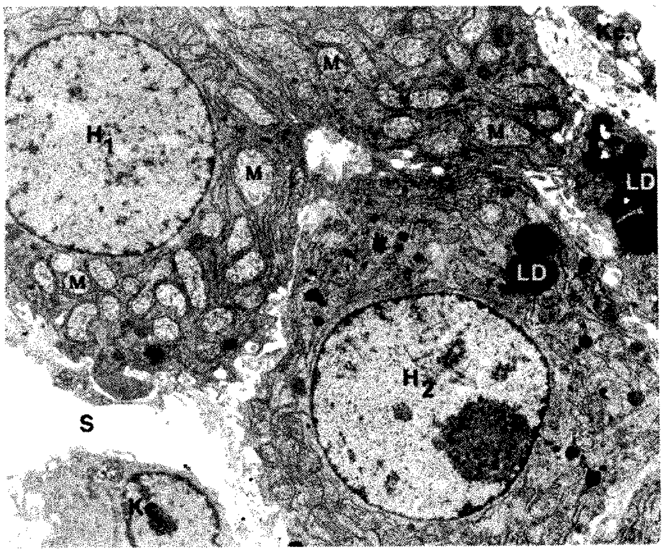

Fig. 2 Foetal hepatocytes after *in utero* exposure to 1.0 mg CH_3HgCl. An increase in lysosomes (L) and lipid droplets (LD) was observed in many hepatocytes. Extrusion of cytoplasmic material from hepatocytes into the sinusoidal spaces was also observed (arrows). Swelling and floccular changes in mitochondria (M) in many hepatocytes (H_1) were observed while mitochondria in some hepatocytes (H_2) appeared normal. (× 4,650).

Electron microscopic examination of foetal hepatocytes revealed, in addition to those changes observed by light microscope, mitochondrial degeneration, occasional accumulations of lipid droplets and dilatation of endoplasmic reticulum. Extrusion of hepatocyte cytoplasmic material into the sinusoidal spaces was also common (Fig. 1). Mitochondrial changes in hepatocytes consisted of an increasing electronlucid floccular matrix, diminution of cristae and occasional swelling (Fig. 2). Similar degeneration of hepatocytes, particularly hepatocyte mitochondria, has been observed in adult rats after methylmercury intoxication[13,14].

Several studies have shown that epithelial cells of renal proximal convoluted tubules accumulate administered mercury in lysosomes and mitochondria[15,16]. Similar accumulation of mercury may be responsible for the hepatic lesions observed under our experimental conditions.

Our results demonstrate a significant causal relationship between maternal exposure to non-teratogenic doses of methyl mercury (1.5 to 3.0 p.p.m. mercury) during gestation and the resulting foetal liver injury. It certainly seems that early gestational exposure of pregnant female rats to minute amounts of methylmercury, which in themselves do not produce maternal neurological symptoms or gross teratology, may constitute considerable risk to the foetus.

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Glutathione-oxytocin transhydrogenase of human placental origin with specificity towards neurohypophysial hormones

THE rapid disappearance of endogenous neurohypophysial hormones from the peripheral circulatory system has led many workers to study the mode of inactivation and site of degradation of these polypeptide hormones. Extracts of kidney[1], liver[2,3], pancreas[4], uterus[5], myometrium[6], ovaries[7] and brain and hypothalamic tissue[8] have all been investigated for their ability to destroy the biological activity of oxytocin and vasopressin.

Of the enzyme systems capable of destroying oxytocin, those associated with pregnancy have been the most extensively studied[9]. Fekete[10] first demonstrated that pregnancy serum inactivated the oxytocic activity found in the posterior pituitary lobe and it was later shown[11] that the inactivation of oxytocin and vasopressin extended to fresh extracts of human placentas. Although the placenta is rich in nonspecific aminopeptidases, the enzyme generally known by the name of oxytocinase is considered to be primarily responsible for the inactivation of oxytocin. This enzyme is a cystine aminopeptidase and causes hydrolytic cleavage of the cystine-tyrosine bond in positions 1 and 2, respectively, of the peptide hormone[12].

Branda and Ferrier[13] have described the biological inactivation of oxytocin and deamino-oxytocin by placental extracts. These workers attributed the inactivation of deamino-oxytocin, which is not attacked by cystine aminopeptidase, to the presence of a thiol-protein-disulphide oxidoreductase followed by the action of tissue peptidases. We have examined placental extracts for a transhydrogenase enzyme system and determined its specificity towards the neurohypophysial hormones.

Placentas obtained from normal vaginal deliveries were cut into small pieces and after washing three times in 0.9% (w/v) saline at 4° C, were homogenised in an equal volume of distilled water using an Ultra Turrax T45 homogeniser (Janke and Kunkel Co., Germany). The particulate matter was removed by centrifugation at 1,400*g* for 10 min and further centrifugation of the supernatant at 105,000*g* for 10 min gave a clear placental extract which was used for enzyme assay using various compounds as substrates. Glutathione-protein-disulphide oxidoreductase activity was measured using the substrate at a concentration of 0.13 mM in Tris-HCl (0.1 M, *p*H 8.0) containing EDTA (1 mM). The assay method was based upon that described by Katzen and Stetten[2] in which NADPH (0.5 mg) is oxidised in the presence of reduced gluthathione (16 mM) and glutathione reductase (5 IU). Enzyme activity is calculated from the change in absorbance min^{-1} at 340 nm over a period of 5–6 min.

The glutathione-protein transhydrogenase activity against different disulphide-containing hormones and compounds is shown in Table 1. Of those proteins used as substrates, oxytocin and [8-arginine]-vasopressin were the most readily attacked whereas insulin was reduced to an extent of only 14% that of oxytocin. Cystine, homocystine and penicillamine disulphide were also readily reduced. As a result of the cleavage of the disulphide bond of oxytocin, we have named this enzyme system placental glutathione-oxytocin transhydrogenase.

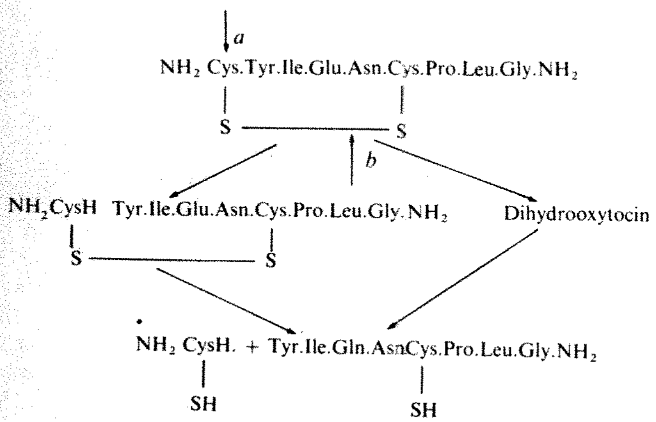
a
NH₂ Cys.Tyr.Ile.Glu.Asn.Cys.Pro.Leu.Gly.NH₂
S
S
b
NH₂CysH Tyr.Ile.Glu.Asn.Cys.Pro.Leu.Gly.NH₂
S
S
Dihydrooxytocin
NH₂ CysH. + Tyr.Ile.Gln.AsnCys.Pro.Leu.Gly.NH₂
SH
SH

Fig. 1 Diagrammatic representation of the possible pathways involved in the enzymatic degradation of oxytocin by human placental tissue. *a*, Attack by cystine aminopeptidase; *b*, attack by placental glutathione-oxytocin transhydrogenase.

Table 1 Activity of placental glutathione-protein transhydrogenase with various protein hormones and compounds containing disulphide bonds

Substrate	Relative activity
Oxytocin	100
(8-arginine)-vasopressin	80
Bovine albumin	56
Human chorionic gonadotrophin	53
Human placental lactogen	48
Insulin	14
Cystine	190
Homocystine	140
N-benzyoxycarbonyl cystine	40
Cystamine 2,2′-dithioethylamine	9
Cystine dimethyl ester	152
Cystine dibenzyl ester	30
Cystine di-*p*-nitroanilide	66
Cystine di-β-naphthylamide	49
Penicillamine disulphide	167
Dithiodioacetic acid	38
Dithiodiproprionic acid	27
Dithiodibutyric acid	32
Dithiodibenzoic acid	38
Dithiodiacetic dibutyl ester	66

The activity is expressed as a percentage of the activity with respect to oxytocin.

Inactivation of vasopressin by the kidney[14] and oxytocin by liver homogenates and tissue extracts[4] is now considered to proceed through a transhydrogenase enzyme system[3]. The enzyme system present in the mammalian liver has been shown to possess a reactivity towards insulin[2] which is 14 times greater than the activity using oxytocin and vasopressin as substrates. In this context it is interesting to note that the liver also elaborates a protease which reacts specifically with insulin[15].

Our findings show that the human placenta is similar to that of the liver in having both a proteolytic and transhydrogenase enzyme system specific towards the same substrate, that is cystine aminopeptidase and placental glutathione-oxytocin transhydrogenase. With the evidence of a transhydrogenase enzyme system being present in the placenta, the role of cystine aminopeptidase as being of prime importance in the degradation of oxytocin is now questionable. Oxytocin may be cleaved by two pathways as illustrated diagrammatically in Fig. 1. Cleavage of the disulphide bond by the transhydrogenase system can occur before peptidase action at the cystine–tyrosine residues or proteolytic action may be the initial step. It has not yet been established which of these sequence of events predominates.

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Structural and crystallographic observations on hagfish insulin

INSULIN is a well defined globular protein made up of two small peptide chains linked together through disulphide bonds[1,2] It contains an unusually high proportion of hydrophobic residues and thus tends to readily self-associate[3 4] In many species the hormone forms dimers in solution and, in the presence of Zn^{2+}[4] ions, hexamers[4 5] Recently, Hodgkin and coworkers[6,7] have determined the crystal structure of the rhombohedral form of porcine Zn-insulin at 1 9 Å resolution[6,7] Two molecules of insulin occur in the asymmetric unit, related through an approximate twofold axis to form a nearly symmetrical dimer Three such dimers in turn associate through coordination of histidine residues B10 with two zinc ions to form a nearly spherical hexamer In spite of the availability of the amino acid sequences of a wide variety of vertebrate insulins[2,8], however, comparative studies on the detailed structure and modes of association of vertebrate insulins have been lacking

We have recently studied the biosynthesis and structure of insulin in a cyclostome, the Atlantic hagfish (*Myxine glutinosa*)[9,10] The cyclostomes represent highly specialised survivors of the earliest vertebrates, the ostracaderms, and thus probably diverged from the gnathostomian vertebrates about 500 Myr ago The islet organ in this species has been studied both structurally and functionally[11 12] Our interest in the associative behaviour of hagfish insulin arose particularly from the early finding, in primary structure analyses, that aspartic acid replaces histidine at position B10 (ref 10) The crystallographic and sequence data presented here suggest that hagfish insulin forms dimers similar in structure to those of other insulins but does not form hexamers

Islet organs were obtained from 4,200 hagfish at the Kristineberg Marine Biological Station (Fiskebackskil, Sweden) Frozen islets were extracted twice with acid-ethanol, and the extract was fractionally precipitated by a modification of the method of Davoren[13] The insulin-containing precipitate was gel filtered over a 115 × 5 cm BioGel P-30 column (BioRad Corporation) in 3 M acetic acid The insulin and proinsulin[14] fractions were identified by means of a double-antibody radioimmunoassay, using a guinea pig anti-hagfish insulin antibody and iodinated hagfish insulin as a tracer The purity of the BioGel fractions was assessed by polyacrylamide gel electrophoresis and by paper and cellulose thin-layer electrophoresis, the insulin (yield about 10 mg) appeared to be at least 90% pure, the major contaminant probably being a desamido component

Unlike most other insulins, hagfish insulin crystallised readily from a dilute acetone-containing citrate buffer near *p*H 6 0 in the absence of zinc Insulin has previously been observed to crystallise in the absence of zinc only in acidic conditions (*p*H 2–4) (ref 15) The crystallisation procedure was a modification of a micromethod used in the Novo Research (Bagsraerd, Denmark) Two solutions were employed (I) 30 ml 1 M HCl, 160 ml acetone and water to 1 l, (II) 0 1 M sodium citrate containing 160 ml acetone l^{-1} When solutions I and II were mixed in the proportions of 5 4, the final *p*H was about 6 0 100 µg of hagfish insulin was placed in a small, conical Pyrex tube, was dissolved in 20 µl of solution I, and was heated briefly in a water bath (50°–55° C) 16 µl of solution II was then added, and the solution was mixed and warmed to 50°–55° C and then was allowed to cool to 25° C over a period of 12 h In these conditions large tetragonal crystals formed at 25° C or at 4° C within 2 d, even in the presence of 0 01 M EDTA Compositional and electrophoretic analysis of washed crystals revealed only hagfish insulin and the putative desamido form

The crystals were clear bipyramids up to 0 5 mm in length X-ray diffraction data on wet mounted crystals showed that these were tetragonal, space group $P4_12_12$ (or the enantiomorph) (ref 16), with cell dimensions of a = 38 51 Å, c = 85 37 Å If the molecular weight of the monomeric protein is taken to be 5,967, then eight molecules of protein in the cell would give as a specific volume V = 2 65 Å^3 per dalton This value is within the range for proteins reported by Matthews[17], but is considerably larger than the 1 9 Å^3 per dalton calculated for rhombohedral Zn-insulin, suggesting looser packing With eight insulin molecules per tetragonal unit cell in $P4_12_12$ or $P4_32_12$, there is one insulin molecule in the asymmetric unit which may form a perfect dimer across the crystallographic twofold axis, but which could not form a hexamer of the type found by Hodgkin *et al* in porcine zinc-insulin crystals We have collected diffraction data to 2 5 Å on these crystals, and are collaborating with Professor Hodgkin's group in Oxford on a complete crystal structure analysis

The methods used in our laboratory for amino acid sequence analysis have been reported elsewhere[18] Compositional data for hagfish insulin have been reported previously[19]

A Chains

	1				5					10				15						20	21
Hagfish	Gly	Ile	Val	Glu	Gln	Cys	Cys	His	Lys	Arg	Cys	Ser	Ile	Tyr	Asn	Leu	Gln	Asn	Tyr	Cys	Asn
Porcine	Gly	Ile	Val	Glu	Gln	Cys	Cys	Thr	Ser	Ile	Cys	Ser	Leu	Tyr	Gln	Leu	Glu	Asn	Tyr	Cys	Asn
Other residues			His	Asp				Ala	Gly Arg Asn	Pro Val Thr		Asp Asn Thr	Arg Lys	Phe His	Asp			Ser			

B Chains

	0	1				5					10					15					20
Hagfish		Arg	Thr	Thr	Gly	His	Leu	Cys	Gly	Lys	Asp	Leu	Val	Asn	Ala	Leu	Tyr	Ile	Ala	Cys	Gly
Porcine		Phe	Val	Asn	Gln	His	Leu	Cys	Gly	Ser	His	Leu	Val	Glu	Ala	Leu	Tyr	Leu	Val	Cys	Gly
Other residues	Val Met	Ala	Pro Ala	Pro Lys Ser Ala	Arg Pro					Pro	Asn			Asp	Thr			Ser			Gln

	21				25					30	31
Hagfish	Val	Arg	Gly	Phe	Phe	Tyr	Asp	Pro	Thr	Lys	Met
Porcine	Glu	Arg	Gly	Phe	Phe	Tyr	Thr	Pro	Lys	Ala	
Other residues	Asp	Asp					Ser Ile Gln	Ser	Met	Ser Thr Asp	(Arg)

Fig 1 The amino acid sequence of hagfish and porcine insulins, with substitutions at each position from other known insulins shown for comparison The additional residue of the hagfish B chain might be the result of either an insertion or deletion event at position 29 or 30 or a single base change within the codon corresponding to the first residue (Arg) of the interchain connecting segment of the porcine proinsulin

Amino acid sequence studies were carried out on the S-carboxymethylated or oxidised chains of hagfish insulin separated by electrophoresis or gel filtration These will be reported in detail elsewhere (J D P, S O E, and D F S, unpublished) The results of these studies provide clues to some aspects of the three-dimensional structure of hagfish insulin A comparison of the sequence of hagfish insulin with that of porcine insulin and the cumulative data on individual amino acid substitutions in all known vertebrate insulins[8] is shown in Fig 1

The most striking feature of the hagfish insulin sequence is that, except at position B18, every residue which is invariant among the other known insulins is also conserved in the hagfish sequence These invariant residues include all of the cysteines and most of the hydrophobic residues present at the dimer interface in porcine insulin crystals (that is, B11, B12, B16, B24-27) The substitution of aspartic acid for histidine at position B10 is an important difference that may account for the absence of hexamers in the hagfish insulin crystals (Substitutions at this position are also known to occur in the insulins of the guinea pig and coypu[8], but crystallisation studies have not been reported Islet tissues from these do not contain much zinc[20]) The substitution of phenylalanine and threonine for the hydrophobic residues usually present at positions B1 and B2 may also interfere with hexamer formation Residues A8 to A10 make up a very basic centre similar to that found in some fish insulins[2] Sequence homologies (Fig 1) suggest that the helical regions of the A chain (A2 to A8 and A13 to A20) are similar in the two species and that the α-helical segment of the B chain (B9 to B19) may also be present in the hagfish

From this analysis there emerges a predicted three-dimensional structure of hagfish insulin which is similar to that of known mammalian insulins Major sequence changes are localised in the extended chain regions, positions A8-A12 and the amino end of the B chain (B1-B10) The stretches of α-helix, the cross bridges, and the residues involved in dimerisation, all of which are important structural determinants in porcine insulin, appear to be conserved in hagfish insulin On the other hand, the zinc binding site and other features that may play a role in stabilising a hexamer structure are not well conserved in hagfish insulin, which is in keeping with the tetragonal symmetry of these crystals These predictions appear to be borne out also by a recent study at 6 Å resolution, comparing the structures of hagfish and porcine insulins[21] The functional significance of these differences in terms of either efficient insulin formation or tissue binding are still not clear The conservation of features related to dimerisation, however, may mean that the dimer is a significant functional entity (Although guinea pig insulin has been reported not to form dimers in solution[22], the necessary regions for dimerisation are present and self-association may occur, for example, at an appropriate tissue-receptor site) Further crystallographic analysis of hagfish insulin combined with studies of its biological activity[19] should help to more clearly define those regions and structural features of the insulin molecule that are necessary for its effective receptor interaction and biological activity[21]

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Changes in histone methylase activity of rat brain and liver with ageing

No function has been found for the methylation of the ε-N-amino group of lysine in certain histones that have been demonstrated in thymus gland, liver and erythrocytes as well as several other organs[1] It has been suggested, however, that this modification of the histone polypeptide effects the DNA-histone interaction and may be involved in regulating gene expression[1] Methylation appears to be very specific, occurring after histone synthesis is complete[2] Methylation of certain histones can be correlated with development Gershey *et al* reported that 3-methylhistidine was formed more rapidly in immature erythroid cells than in mature erythrocytes[3], and Borun *et al* found that in developing HeLa cells the lysyl residues of F_{2b}, F_3 and F_{2a} histones were methylated mainly in the G_2 phase and during mitosis[4] Methylation of rat brain histones also seems to depend on age After the intraperitoneal injection of L-$^{14}CH_3$-methionine into different age classes of rats we found that only trace amounts of ^{14}C-methyl were incorporated into brain histones after maturity[5] If L-^3H-lysine and L-$^{14}CH_3$-methionine are administered to newborn rats biphasic type decay curves (^3H and ^{14}C) are obtained for all

Table 1 Age dependence of histone methylation in rat brain and liver nuclei

	Specific activity of total histones			
	Brain		Liver	
	d p m mg^{-1}	pmol mg^{-1}	d p m mg^{-1}	pmol mg^{-1}
Newborn	142,000	65	65,000	29
11	260,000	118	51,000	23
21	179,000	81	72,000	32
83	90,000	40	—	—
365	81,000	36	45,000	20
900	26,100	12	28,000	13

Nuclei from 15 g of brain or liver from different age groups were isolated and suspended in 0 32 M sucrose containing 0 1 M potassium phosphate buffer, *p*H 7 5 and 1 0 mM $MgCl_2$ The nuclei were incubated with 100 µCi C^3H_3-S-adenosylmethionine (1 0 Ci mol^{-1}) for 30 min at 37° C The reaction was terminated by chilling to 0° C and the histones purified as previously described[6] The histones were precipitated with acetone and the protein concentration and radioactivity were determined

histone subfractions During brain development (up to 50 d) the arginine-rich histones have a halflife of 32 d while no turnover is observed after maturity as measured by either ^{3}H or ^{14}C decay We now find that the level of the histone methylases and the ability of brain histones to accept methyl groups decreases during ageing Freshly prepared brain and liver nuclei from rats of various ages were incubated with C^3H_3-S-adenosylmethionine The reaction mixtures were chilled to 0° C, the nuclei centrifuged down and extracted three times with 0 14 M NaCl The residue was washed with methanol–chloroform, acetone, acetic acid (*p*H 3 0) and the histones extracted with 0 4 M HCl[6] When L-^{14}C-lysine was added to the reaction mixture no ^{14}C was incorporated into the resultant histones excluding *in vitro* synthesis of histone in our system

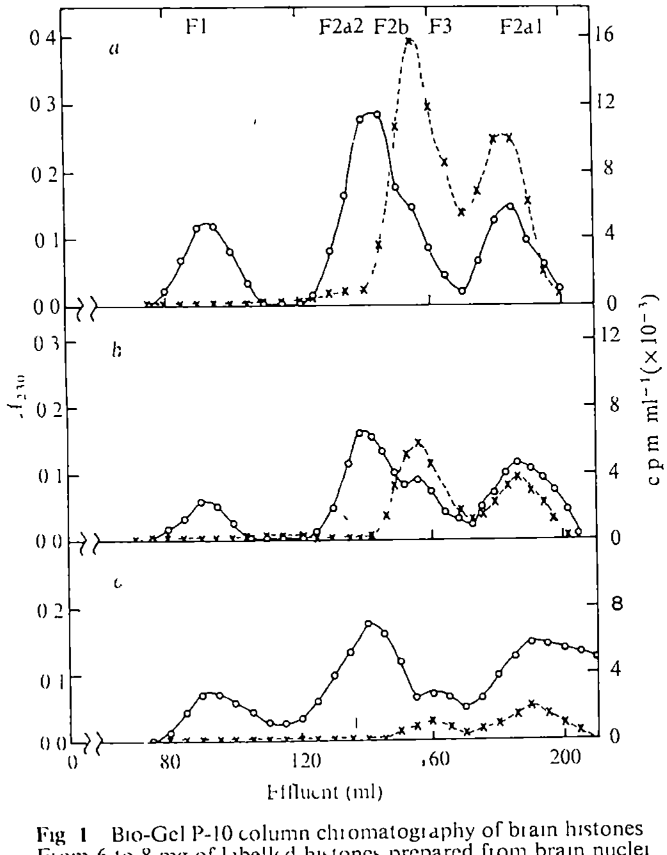

Fig 1 Bio-Gel P-10 column chromatography of brain histones From 6 to 8 mg of labelled histones prepared from brain nuclei as outlined under Table 1 were dissolved in 10 mM HCl containing 6 M urea This solution was applied to a Bio-Gel P-10 column (1 6 350 cm) and the histones eluted with 10 mM HCl Fractions of 3 5 ml were collected Samples of 0 2 to 1 0 ml were taken from each tube for the determination of radioactivity Histones were from newborn rats (*a*), 83-d-old rats (*b*) and 900-d-old rats (*c*)

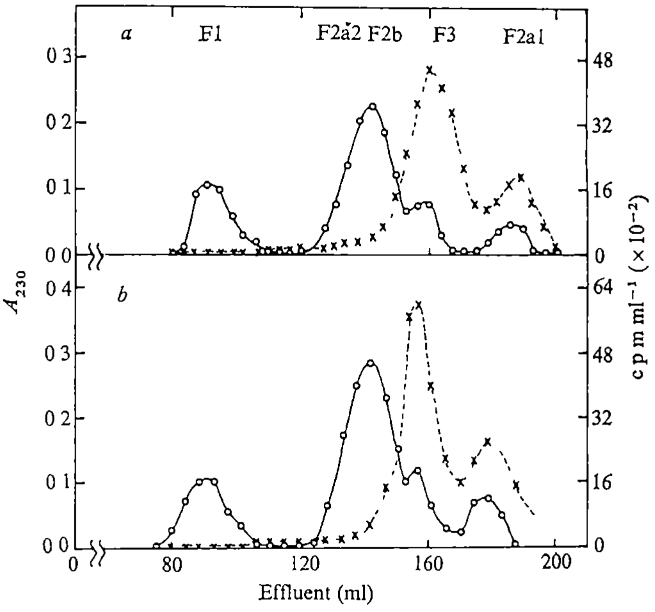

Fig 2 Bio-Gel P-10 column chromatography of liver histones from newborn rats (*a*) and 900-d-old rats (*b*) The labelled histones prepared from liver nuclei (Table 1) were fractionated on Bio-Gel P-10 as outlined under Fig 1

There was an increase in methylation of the histones in brain nuclei during the first few days after birth (Table 1) After approximately 11 d the activity decreased progressively throughout the life of the animal The extent of methylation of histones in intact brain nuclei was three to four times greater during the various developmental stages than in the liver nuclei, while the decrease in activity with ageing was more pronounced in brain nuclei

After fractionation on Bio-Gel P-10 the F_3 and F_{2a1} histones were the only components from brain and liver nuclei found to be highly methylated (Figs 1 and 2) The amount of labelled methyl groups incorporated into the F_3 and F_{2a1} histones decreased during ageing in both liver and brain while the degree of methylation of F_3 was always greater than that of F_{2a1}

After hydrolysis, the products of methylation in the F_{2a1} and F_3 histones were chiefly monomethyllysine and dimethyllysine (Table 2) No methylated arginine or histidine was found in any of the histones analysed In all cases, except the F_{2a1} from liver nuclei of 2 5-yr-old rats, there was more monomethyllsine than dimethyllysine By direct quantitative analysis we find that 8 1% of the lysyl residues in rat liver F_{2a1} histones are methylated, mainly as ε-N-dimethyllysine In F_3 methyl groups are present on 13 4% of the lysyl residues mainly as mono and trimethyllysine in a ratio of 1 1 Tidwell *et al* have also found that ε-N-dimethyllysine is the predominant form in F_{2a1} from rat liver[2] The disproportionately high content of monomethyllysine in F_{2a1} and F_3, as measured by pmol ^{3}H incorporated (Table 2), would be expected if the methyl groups were added sequentially

Table 2 Distribution of methylated lysyl residues in liver histones

Histones	pmol mg^{-1} histone		
	Monomethyllysine	Dimethyllysine	Trimethyllysine
F_3 (newborn)	20 64	8 30	0 70
F_3 (2 5-yr-old)	14 42	5 39	0 19
F_{2a1} (newborn)	16 23	2 48	0 05
F_{2a1} (2 5-yr-old)	5 59	11 70	0 00

The labelled liver histones fractionated on Bio-Gel P-10 (Fig 1) were hydrolysed in 6 0 M HCl for 24 h at 110° C and chromatographed on a column of Beckman PA-35 resin as described previously The level of methylated lysine was calculated from the amount of ^{3}H present under the peaks

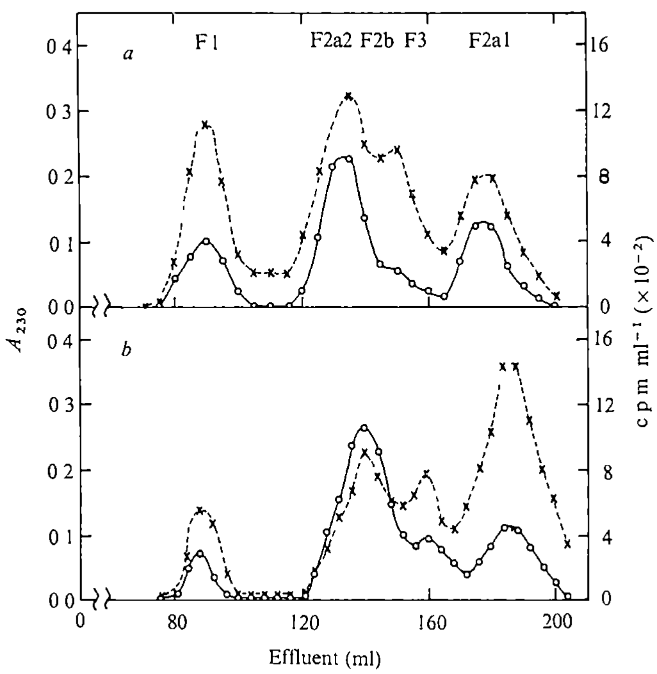

Fig 3 Bio-Gel P-10 column chromatography of brain (*a*) and liver (*b*) histones after incubation with methylases from the same organ Brain histones from 11-d-old rats and liver histones from 21-d-old rats were incubated with methylases from the same organ of 2 5-yr-old animals in the presence of C^{3}H$_3$-S-adenosylmethionine as described under Tables 3 and 4 The histones were fractionated on Bio-Gel P-10 as described under Fig 1

The apparent decrease in methylation of brain and liver histones with age could be due to a decrease in the level of histone methylases and/or the availability of sites on the histones to accept methyl groups To delineate between these two possibilities, histone methylases were assayed in young and old animals using as substrates histones prepared from the same organs

As Tables 3 and 4 show, the histone methylase was considerably more active in extracts of liver and brain nuclei of young animals There was no significant difference in the ability of histones prepared from liver nuclei of young or old animals to accept methyl groups Histones prepared from the brains of young rats were somewhat better methyl group acceptors than those from old brains Our data from both intact nuclei and soluble enzyme preparations agree with those of Paik and Kim[8], who found that histone methylase III, prepared from rat brain, increased after birth until the 10th day and decreased significantly thereafter They used calf thymus histone as substrate and would not have observed differences in the acceptor ability of histones prepared from animals of different ages

Table 3 Histone methylase activity in the brain of young and old rats

Methylase source 1 0 mg	Histone source 1 0 mg	Specific activity d p m mg^{-1}	S-adenosyl-methionine utilised pmol mg^{-1}
Old	Old	11,200	50
Old	Young	17,500	79
Young	Old	24,500	111
Young	Young	32,400	149

The enzyme fractions and histones were prepared from 11 and 900-d-old rats[7] The reaction mixtures contained 50 μCi C^{3}H$_3$-S-adenosylmethionine, 20 mM phosphate buffer *p*H 7 5, 1 0 mM dithiothreitol and 1 0 mg each of histone and enzyme protein from either old or young animals After incubation for 30 min at 37 C the reaction was stopped by adjusting the *p*H to 9 7 with NaOH and the addition of urea 8 M[6] The reaction mixtures were applied to DEAE-cellulose columns (1 0×20) previously equilibrated with 10 mM glycine NaOH buffer, *p*H 9 7, containing 8 M urea The histones were eluted with the same buffer, dialysed against 10 mM HCl and lyophilised The histones were heated at 70 C for 20 min in 1 1 M trichloroacetic acid (TCA), collected on Millipore filters and washed with 1 1 M TCA The precipitates were suspended in 10 ml Bray's counting fluid[9] and the radioactivity was determined using a Packard liquid scintillation counter

Table 4 Histone methylase in the livers of young and old rats

Methylase source 1 0 mg	Histone source 1 0 mg	Specific activity d p m mg^{-1}	S-adenosyl-methionine utilised pmol mg^{-1}
Old	Old	7,130	32
Old	Young	6,100	28
Young	Old	22,760	102
Young	Young	25,000	116

The enzyme fractions and histones were prepared from 21 and 900-d-old rat liver nuclei All other conditions were the same as outlined under Table 3

The labelled histones from our experiments were fractionated on Bio-Gel P-10 and the distribution of methylated components was determined The profiles of brain and liver histones from young animals methylated by the enzyme from old animals are given in Fig 3 All histone subfractions were highly methylated When methylases from brain and liver nuclei of young animals were used the resultant specific activities of the histones were greater but the distribution of label in the subfractions did not change

The distribution of labelled methyl groups in these histones differed significantly from what we observed in experiments with intact nuclei Apparently when the histones are associated with the DNA few, if any, of the lysyl residues on the F_1, F_{2a2} or F_{2b} histones are accessible to the methylases Apparently the specificity for the methylation of the lysyl residues in the polypeptide chain is lost when histones are dissociated from DNA These experiments suggest that some of the specificity for methylation resides within the arrangement of the histones on DNA and that methylation occurs after the histones are bound to the DNA

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Immunological distinction between the possible origins of enzymatic activity in a polypeptide fragment of staphylococcal nuclease

WE have used an immunological approach to demonstrate that the residual enzymatic activity in a large polypeptide fragment of staphylococcal nuclease is an intrinsic property of the fragment and not an artefact due to contamination with intact enzyme These results are consistent with present concepts of the folding of polypeptide fragments to form structures similar to the native protein and may offer a method for the investigation of other macromolecular systems

Staphylococcal nuclease consists of a single polypeptide chain of 149 amino acids[1] and has nucleolytic activity towards DNA which is readily measurable spectrophotometrically After trifluoroacetylation to block free amino groups, nuclease can be cleaved by trypsin preferentially at arginine residue 126 (ref 2) Removal of the blocking groups and subsequent chromatographic purifications yield a polypeptide fragment consisting of amino acid residues 1 to 126 (previously designated (1–126) (ref 3, 4)) This fragment contains all amino acid residues of known importance to the active site Spectral measurements of (1–126) indicate an absence of the characteristic ordered secondary structure of nuclease, which suggests the importance of long range tertiary interactions of the distal fragment in the proper folding of the native nuclease molecule[4]

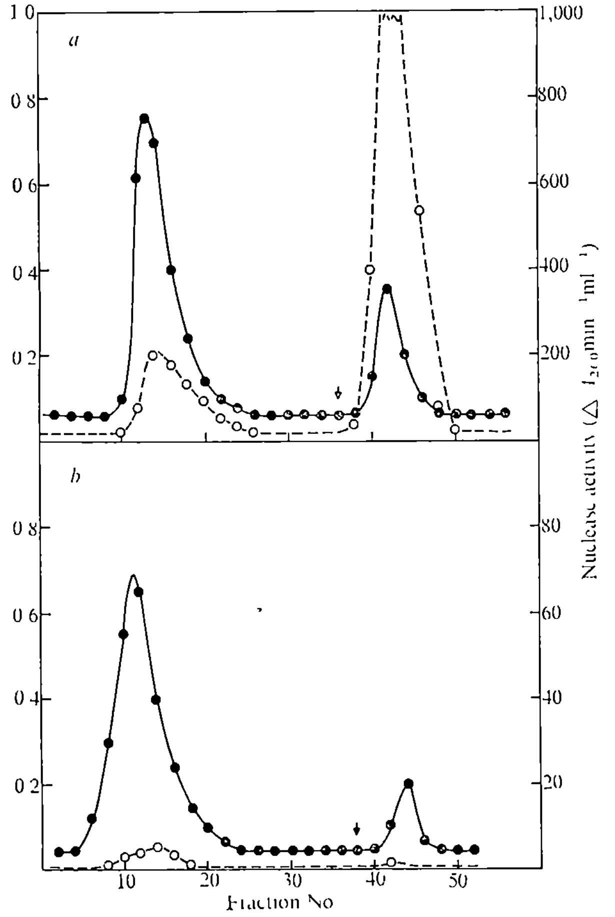

Fig 1 Affinity chromatography of nuclease (*a*) and of (1–126) (*b*) on Sepharose columns bearing the substrate analogue thymidine diphosphate[5] Point of buffer change to effect elution is indicated by arrows in both figures ●–●, Optical density, ○–○, enzymatic activity Note that the acid eluate in (*a*) has the full activity of pure nuclease (a small amount of active material is not bound in these conditions), while the 1–126 adsorbed to the column is of the same activity as that which passes through

But, (1–126) preparations are not devoid of enzymatic activity affinity chromatography on pdTp-Sepharose[5] generally yields a relatively inactive run-through peak followed by pure, active nuclease in the acid-eluted peak (Fig 1*a*) Chromatography of (1–126) preparations yielded peaks in which both the run-through and acid-eluted peaks showed a similar activity/protein ratio (Fig 1*b*) Multiple passages of the first peak through the affinity column decreased the activity of this material to a level about 0 11% that of nuclease, on a molar basis, and which could not be decreased by further passages It is important to know whether this activity is due to contaminating native nuclease or is intrinsic to (1–126) Such intrinsic activity would imply that the correct folding of this fragment to generate an enzymatically active site must occur spontaneously, albeit to a small extent, even in the absence of the tertiary stabilising influence provided by residues 127–149 Unfortunately, most analytical methods do not distinguish between these explanations for the activity of (1–126) The amount of contaminating nuclease that would suffice to produce 0 11% enzymatic activity is too small to detect by analytical gels or by the unique spectral characteristics of nuclease Conversely, the minute fraction of folded (1–126) that might be sufficient to generate this level of enzymatic activity would not be detected spectroscopically

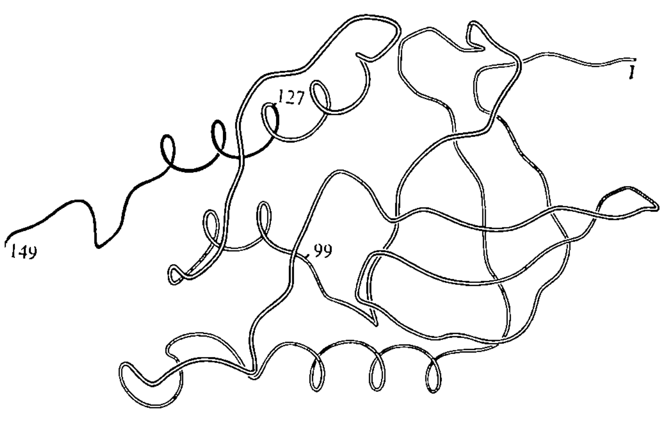

Fig 2 An artist's representation of nuclease with the sequence between amino acids 127 and 149 shaded to indicate the molecular localisation of the antigenic determinant of anti-(127–149)$_{N}$ antibodies

We have therefore used an immunological approach We prepared and fractionated antibodies to nuclease as previously described[6] Briefly, antibodies to the active enzyme were fractionated on Sepharose columns to which selected fragments of nuclease had been bound covalently The antibody fraction resulting from absorption to and elution from Sepharose-(99–149) and then Sepharose-(127–149) has been called anti-(127–149)$_{N}$, the subscript N indicating that the antibodies were prepared by immunising with intact native nuclease Such antibodies combine with nuclease to produce an enzymatically inactive antibody–antigen complex[7] But, since the reaction presumably involves an antigenic determinant in the region 127–149 of the nuclease molecule (Fig 2), such antibodies ought not to react with (1–126)

The effects of anti-(127–149)$_{N}$ antibodies on the activities of nuclease and (1–126) are illustrated in Fig 3 An amount of antibody (5 μg) sufficient to inactivate 12 5 ng of nuclease did not inhibit the activity of 5 μg of (1–126), which contained comparable enzymatic activity As a control against the possibility that the larger amount of protein in the latter case might have interfered with the antibody-antigen interaction, the effect of anti-(127–149)$_{N}$ on a mixture of nuclease and (1–126) was also studied (Fig 3) The antibodies were found to reduce the activity of such mixtures to that which would be predicted for the amount of (1–126) present

Our results indicate then, that the residual enzymatic activity associated with polypeptide fragment (1–126) is an intrinsic property of that fragment and not due to contamination with nuclease We have shown previously that many features of the interactions of purified monospecific anti-nuclease antibodies with fragments of nuclease can be interpreted by a model in which the fragments are presumed to exist in solution in conformational equilibrium between various disordered or random conformations and the conformation assumed by the corresponding amino-acid sequence in the native protein[8] Our present results can also be interpreted by such a model, since a consequence of the native folding of the polypeptide fragment (1–126) would be the native orientation of amino acids of the enzymatic active site, leading to a level of residual activity related to the folded fraction The existence of fragments sufficiently folded to yield activity but with altered K_m and V_{max} values might of course affect the numerical relationship between activity and folding

The hypothesis that the primary sequence of a protein determines its native three-dimensional structure has been substantiated for various proteins[9] However, the mechanism by which the primary sequence is translated into correct folding of a protein is unclear Preferred conformations in terms of φ and ψ angles have been demonstrated at the level of tripeptides[10], and the suggestion that proteins may fold sequentially as they are assembled at the ribosome[11] has been discussed widely The demonstration that antibodies to β-galactosidase can bind to incomplete, ribosome-bound protein fragments has been taken as support for this hypothesis[12] But, the apparent requirement of nearly the entire sequence of most proteins before a significant amount of spontaneous folding occurs has been interpreted as evidence for the importance of long-range tertiary interactions in the folding process[3]

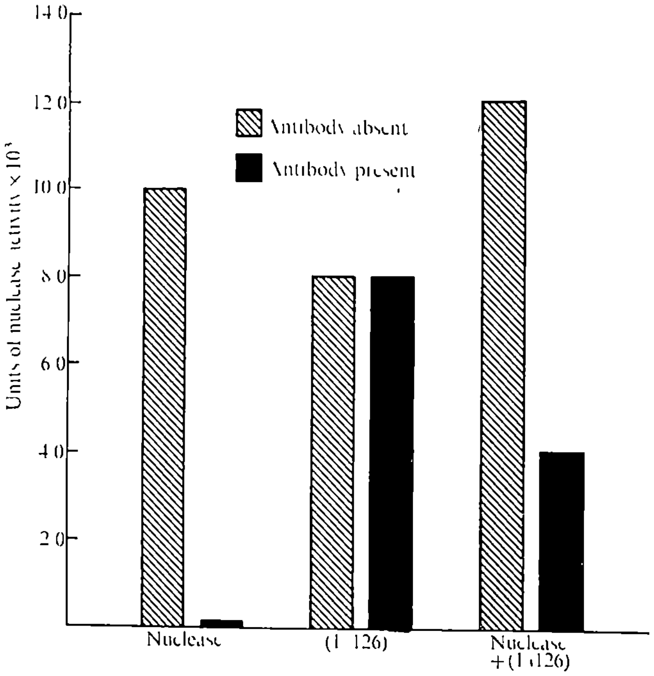

Fig 3 Inhibition of enzymatic activity by anti-(127–149)$_{\text{N}}$ antibodies The first two pairs of bar graphs illustrate samples of nuclease (12 5 ng) or (1–126) (5μg) containing comparable levels of enzymatic activity which were incubated with either saline (control) or with anti-(127–149)$_{\text{N}}$ antibodies (5 μg), and where then assayed for residual enzymatic activity The final pair of bar graphs show the effect of the same amount of antibody on the activity of a mixture of one-half of each of the previous amounts of nuclease and of (1–126) The increased activity of the mixture over that expected from the components may indicate that the complementary sequence of nuclease can affect the folding equilibrium of (1–126), perhaps similar to the folding 'induced' by the fragment (99–149)[4] Antibody reduced the activity of the mixture to a level compatible with the amount of (1–126) in the mixture

The two hypotheses are not necessarily contradictory if one considers polypeptide chains to be undergoing conformational equilibria, since the detection of folded forms depends on the sensitivity of the method of study relative to the position of the equilibrium In terms of mechanism, polypeptides, either on or off ribosomes, can be envisaged as continually trying out different possible three-dimensional conformations The degree to which native format is achieved by an incomplete protein fragment might then be considered a reflection of the relative thermodynamic stability of this conformation as against that of other possible conformations of the same polypeptide chain In the intact protein or in a fairly large polypeptide fragment of that protein, such as (1–126), the ability of the native conformation of the polypeptide sequence to interact with distant portions of the molecule might be expected to increase the thermodynamic stability of that particular conformation Clearly there are many possible three-dimensional conformations of a polypeptide chain of 126 amino acids Thus even the small percentage folding which we propose as the explanation for the intrinsic enzymatic activity of (1-126) represents a considerable restriction of possible conformations of this polypeptide Presumably the sequence of (1–126) permits some, but not all, of the tertiary interactions which lend stability to the native conformation of the intact protein

There are many examples, especially among polypeptide hormones (for example ref 13), where biological activity may be retained in fragments produced by cleavage or synthesis The use of antibodies with specificity for limited regions may help clarify the mechanisms of these phenomena

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Haemoglobin Icaria, a new chain-termination mutant which causes α thalassaemia

The human haemoglobin (Hb) variant Constant Spring (Hb CS) occurs in a number of racial groups and is involved in the aetiology of as many as 50% of all cases of Hb H disease, a disorder which presents a major public health problem in south-east Asia[1,2]. It has an elongated α chain of 172 residues which has probably arisen by mutation of the normal α-chain-terminating codon (UAA) at position 142 to a glutamine codon (CAA), allowing read-through into extrastructural codons at the 3′-end of the α mRNA[3]. We report here the characterisation of Hb Icaria, a second Hb with an elongated α chain of 172 residues. Its structure is identical with that of Hb CS except that residue α142 is lysine instead of glutamine.

The variant, which comprised 0·8% of the total Hb, was discovered during the haematological investigation of a woman from the Aegean island of Icaria. Examination of a haemolysate on starch-gel electrophoresis at *p*H 8·5 showed two minor slow-moving haemoglobin components, one cathodal and the other anodal. Comparison with a haemolysate containing Hb CS showed that both minor bands in the Hb Icaria sample were more cathodal than the corresponding bands of Hb CS (Fig. 1).

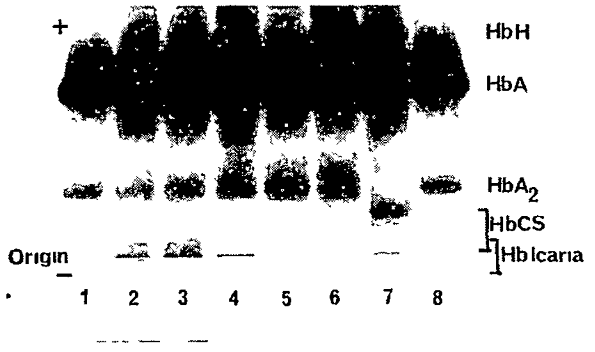


Fig. 1 Starch-gel electrophoresis (Tris-EDTA-borate system, *p*H 8·5, benzidine stain) of red-cell lysates prepared from the following (left to right): 1, normal adult; 2 and 3, Hb Icaria heterozygote; 4 and 5, normal adult; 6, Hb CS heterozygote; 7, individual with Hb H disease and Hb CS; 8, normal adult.

The minor components of Hb Icaria were purified on Amberlite IRC-50 by a procedure similar to that used for the isolation of Hb CS (ref. 3); they remained tightly bound to the top of the column after the elution of Hb A at 25° C with Developer 2. Hb CS, by comparison, remained on the column as a more diffuse band. Both haemoglobins were eluted as a sharp zone by increasing the salt concentration of the Developer 2 buffer to 0·5 M with NaCl. Starch-gel electrophoresis of the purified haemoglobins revealed two major slow-moving components, together with variable amounts of contaminating Hb A. In some preparations it seemed that considerable degradation of the Hb Icaria had taken place, since the mobilities of the slow-moving components were more anodal than in the original haemolysate. This behaviour is similar to that seen occasionally during the purification of Hb CS, and is attributed to degradation of the α-chain 'tail' by red-cell proteases during the purification procedure[3].

A sample of purified Hb Icaria, containing about 30% Hb A, was converted to globin and fractionated by chromatography on CM-cellulose in 8 M urea at *p*H 6·8 (ref. 4). Two major basic α-chain components were observed in addition to the α(A) chain from the contaminating Hb A. Compared with the elution pattern from a sample made from impure Hb CS it was obvious that the minor α-chain components of Hb Icaria were relatively more basic than those of Hb CS, reflecting the differences seen on starch-gel electrophoresis. The abnormal α-chain fractions were combined and recovered by dialysis against 0·5% HCOOH and freeze drying, and then digested with trypsin. Peptide maps of the digest were prepared by high-voltage electrophoresis at *p*H 4·7, followed by chromatography in *n*-butanol:acetic acid:water:pyridine (15:3:12:10 by volume) (ref. 4). A digest of authentic α(CS) chain was also run as a control. Comparison of the two (Fig. 2) revealed that all the normal α(A)-chain peptides were present and that of the three major peptides unique to the α(CS)-chain[3], peptides Y and W$_I$ (that is, those representing residues 154 to 172) were present on the α(Icaria)-chain chromatogram, and had compositions identical to the equivalent peptides eluted from the α(CS)-chain peptide map. The remaining peptide X′ (X in the α(CS)-chain digest, corresponding to residues 142 to 153) was displaced on the α(Icaria)-chain peptide map, being slightly more cathodal on electrophoresis and faster in chromatography. In addition, two new spots, U and V, were observed in the α(Icaria)-chain digest. V had the same mobility in electrophoresis as α3 but was slower in chromatography, while U was slightly slower in electrophoresis than the free lysine (α8). It was also observed that α2, which normally gives a brown/grey colour with ninhydrin, was stained blue.

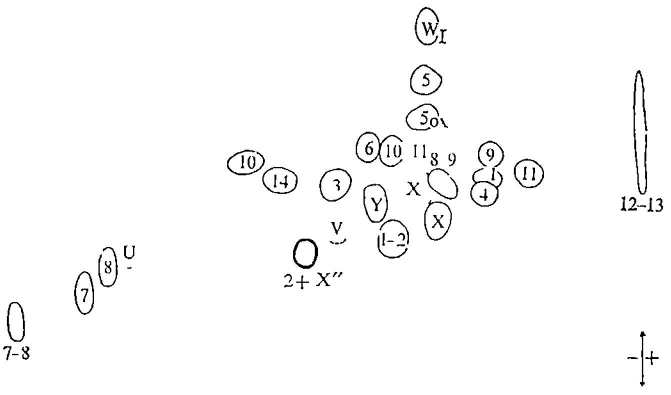


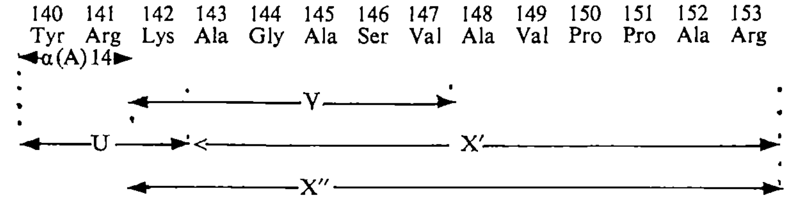
Fig. 2 Superimposed tracings of peptide maps of α(CS) and α(Icaria). Peptides common to both α chains are drawn in solid lines, those unique to α(Icaria) are dotted.

Peptides X′, V, U and 'α2' were cut out and the paper pieces washed with acetone before the peptides were eluted with 6 N HCl. One tenth of each sample was removed, dried and reacted with dansyl chloride for determination of the N-terminal residue[5] and the remainder was hydrolysed for 16 h for amino acid analysis. The results are summarised in Table 1.

X′ has a composition similar to α(CS)X, except for the absence of the glutamine N-terminal residue and the presence of N-terminal alanine. Peptide U, which has N-terminal tyrosine, has the composition of α(A)14 (Tyr, Arg) plus an additional lysine residue. 'α2' is obviously a mixture of genuine α2 (Thr, Asn, Val, Lys), and a peptide(X″) of composition Ser, Pro_2, Gly, Ala_4, Val_2, Arg, Lys, that is, X′+N-terminal lysine. These data are consistent with a sequence ... Tyr-Arg-Lys-Ala ... for residues 140–143 (Table 1) instead of the ... Tyr-Arg-Gln-Ala ... characteristic of all the examples of Hb CS yet studied[2,3] (Hb CS is found occasionally in Greece and was originally called Hb Athens[2]; we have redetermined the structure of Hb Athens and confirmed that its structure is identical with Hb CS.) Furthermore, peptide V has a composition and N-terminal residue consistent with the sequence of residues 142 to 147 (Table 1). In view of the extensive degradation of some of the finally purified samples of Hb Icaria, it seems likely that this represents a truncated version of the α(Icaria) chain, comparable to the CS2 and CS3 derivatives[3] of Hb CS which are thought to be produced by proteolysis during purification of the haemoglobin.

Table 1 Amino acid compositions and sequences of α(Icaria)-chain peptides

	α(Icaria)-chain peptides				αCS-chain peptides	
	X′	U	V	‘α2’* (α2+X″)	α2	α(CS)X
Lys		(0 9) 1	(0 7)†1	(1 7)†2	1	
His						
Arg	(0 8) 1	(0 9) 1		(0 9) 1		1
Asx				(1 0) 1	1	
Thr				(0 9)†1	1	
Ser	(1 0) 1		(0 8) 1	(0 9) 1		1
Glx						1
Pro	(1 7) 2			(1 5) 2		2
Gly	(1 0) 1		(1 0) 1	(1 1) 1		1
Ala	(3 6)†4		(2 0) 2	(4 0) 4		4
Cys						
Val	(1 9) 2		(0 7) 1	(3 0) 3	1	2
Met						
Ileu						
Leu						
Tyr		(0 5)†1				
Phe						
Trp						
N-terminal by DNS-Cl	Ala	Tyr	Lys	Thr+Lys	Thr	Glx (Gln)



* Corrected for ratio of X″ to α2 of 1 3/1
† Yield unreliable due to reaction of N-terminal with ninhydrin

There is now good evidence that human α mRNA has a stretch of at least 100 nucleotides between the chain-termination codon and the poly(A) tract at the 3′-end[3] From a comparison of the amino acid sequences of Hb Constant Spring[3] and Hb Wayne[6] (a frame-shift mutant with an elongated α chain of 146 residues) a unique sequence of 26 nucleotides for codons 140–148 can be deduced for α-chain mRNA in which the normal chain-termination codon at position 142 is UAA (Fig 3) A single base substitution in this codon to CAA (Gln) or AAA (Lys) can thus account for the formation of elongated α-chain variants like Constant Spring and Icaria, translation continues beyond codon 141 until the first in-phase termination codon is reached, presumably at position 173

Hbs CS and Icaria are both present in extremely low amounts, of the order of 0 5 to 1%, in the red cells of heterozygotes In the case of Hb CS, biosynthetic experiments have shown that this is unlikely to be due to a translational defect (such as a rate-limiting step at the glutamine-142 codon) during assembly

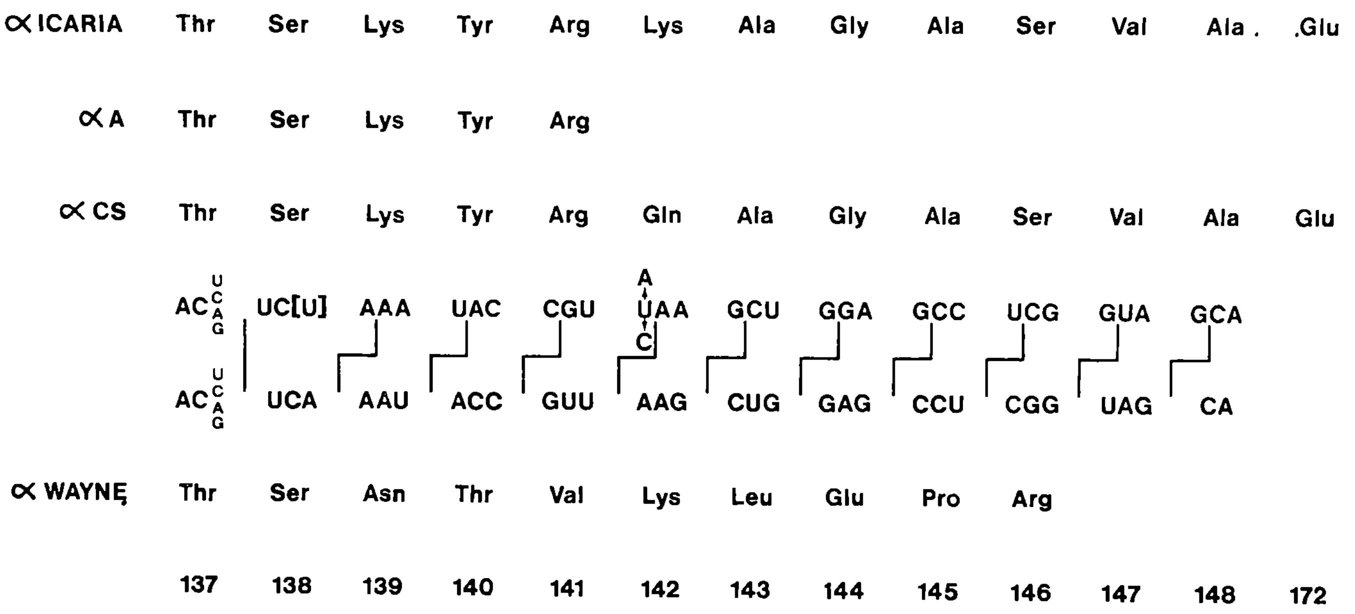

α ICARIA Thr Ser Lys Tyr Arg Lys Ala Gly Ala Ser Val Ala . .Glu
α A Thr Ser Lys Tyr Arg
α CS Thr Ser Lys Tyr Arg Gln Ala Gly Ala Ser Val Ala Glu
A
AC U/C/A/G UC[U] AAA UAC CGU UAA GCU GGA GCC UCG GUA GCA
C
AC U/C/A/G UCA AAU ACC GUU AAG CUG GAG CCU CGG UAG CA
α WAYNE Thr Ser Asn Thr Val Lys Leu Glu Pro Arg
137 138 139 140 141 142 143 144 145 146 147 148 172

Fig. 3 Partial amino acid and nucleotide sequences for α chains of haemoglobins A, CS, Icaria and Wayne The base sequences for codons 138 and 139 have been chosen arbitrarily Deletion of any one of the four bases before codon 140 produces the nucleotide sequence which defines the amino acid sequence at the C-terminal end of the α chain of Hb Wayne In this diagram the third base of the codon for Ser 138 has been deleted, the base sequence of the α mRNA is thus uniquely defined from codon 140 to the second base of codon 148

of the α(CS) chain[3] Rather the evidence suggests that α(CS) mRNA may be much more unstable than normal α(A)mRNA since its activity in the cell cytoplasm seems to be dependent on continued nuclear mRNA synthesis (to be published)

Laux *et al* have recently demonstrated the potential existence of a large base-paired loop, which includes the chain-termination codon, in human α mRNA[7] In phage and ribosomal RNAs such double stranded regions are thought to contribute to the overall stability of the RNA molecules[8,9] Hbs CS, Wayne and Icaria are all produced in very low amounts and are exceptional in that they share the property that they arise by the translation of stretches of mRNA that normally remain untranslated If these regions of the mRNA are essential for its stability or functional integrity, then their unfolding during translation may render the α mRNA more susceptible to degradation by nuclease attack, thus leading to low levels of functional mRNA

There is now strong genetic and biosynthetic evidence that the Hb CS mutation results in an overall deficit of α chains in affected red cells[10], and that its interaction with the severe α-thalassaemia gene (α-thal$_1$) causes about half the cases of Hb-H disease in south-east Asia[2,11] Preliminary studies indicate that Hb Icaria also produces a disorder similar to α thalassaemia The discovery of a further chain-termination variant causing α thalassaemia suggests that inefficiently synthesised Hb variants of this type may account for other forms of thalassaemia Obviously the blood of all thalassaemic patients should be screened carefully for trace amounts of haemoglobin variants

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Measurements of unwinding of *lac* operator by repressor

We report here measurements of the rates of association and dissociation of the *lac* repressor and covalently closed λp*lac* DNAs of various degrees of superhelicity, from which we deduce that the binding of repressor unwinds the *lac* operator by 40° to 90° This small unwinding angle is inconsistent with binding mechanisms involving considerable disruption of DNA base-pairs and, in particular, with the model first proposed by Gierer[1] for specific repressor–operator interactions Our measurements were prompted by the existence of a twofold symmetry in the base sequence of the *lac* operator, which is a prerequisite of the Gierer model The minimal base sequence of the *lac* operator determined by Gilbert and Maxam[2], is depicted in Fig 1 as a regular double helix (Fig 1*a*), and as a Gierer-type structure (Fig 1*b*) According to Gierer's model, the *lac* repressor interacts specifically with the hairpinned structure shown in Fig 1*b*

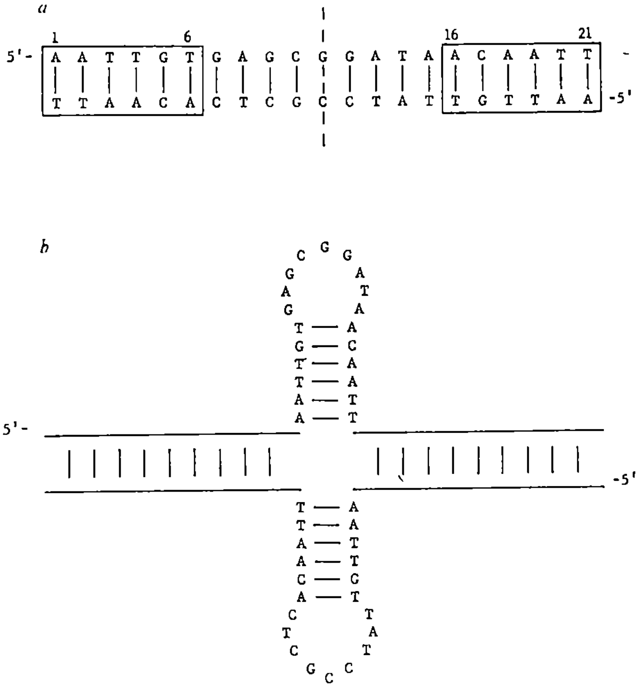
a
1
6
16
21
5'-
A A T T G T G A G C G G A T A A C A A T T
T T A A C A C T C G C C T A T T G T T A A
-5'
b
5'-
-5'

Fig 1 *a*, The minimal base sequence of the *lac* operator taken from ref 2 The boxed regions are the symmetrical hexameric sequences which form the hydrogen-bonded regions of the two stems shown in *b*

The conversion of the *lac* operator from the structure shown in Fig 1*a* to the one shown in Fig 1*b* reduces the number of helical turns between the two complementary strands by approximately two full turns If the twofold symmetry in base sequence extends beyond the published sequence for the operator[2], the short stems shown in Fig 1*b* could be lengthened further, with an even greater reduction in the number of helical turns between the complementary strands For a negatively superhelical DNA, a reduction in the number of helical turns between the complementary strands, which decreases the absolute number of superhelical turns, is favoured thermodynamically[3] Thus for the binding of the *lac* repressor to two otherwise identical DNAs containing the operator, but with only one of the two DNAs containing negative superhelical turns, a direct prediction from the Gierer model is that the affinity of the repressor to the negatively superhelical DNA should be orders of magnitude higher than that to the DNA without superhelical turns[4] Maniatis and Ptashne[5] have used this approach to study the binding of the λ repressor to its operators, and have concluded that it is highly unlikely that the λ operators form some structure other than a linear duplex

Techniques have been developed for studying the specific interaction between the *lac* repressor and operator[6,7] The binding constant can be obtained from either equilibrium[6] or kinetic measurements[7,8] The high precision of the kinetic method makes it the method of choice in determining whether the binding of the *lac* repressor significantly unwinds the operator

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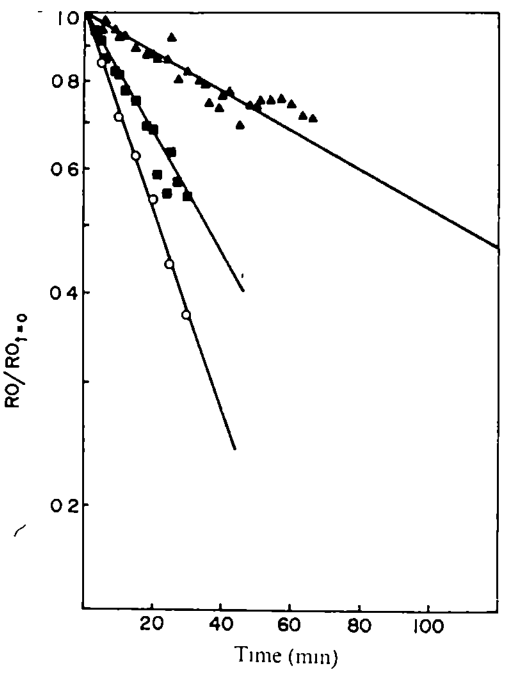

Fig 2 Rates of dissociation of repressor–operator complexes formed with operator DNAs with various degrees of superhelicity. The *lac* repressor was isolated from an *E. coli* strain, BMH461 (ref. 9), carrying a mutation (i^q), which leads to overproduction of the repressor. The purification procedure (to be published elsewhere), which includes DNA-cellulose chromatography, yields consistently, very pure and active repressor. The DNA was extracted from the λp*lac*5 phage purified from a low-thymine-requiring lysogenic strain MBC5 ((*lac pro*$_{AB}$) $\Delta_{XIII}T^-B^-_1$ nal$_A$R (λ $C_{1857}S_7$ p*lac*5 $i^-o^+z^+y^-$)) after growth in the presence of ^{3}H or ^{14}C-labelled thymine. The cyclisation and the covalent closure of the DNA in the presence of various amounts of ethidium bromide were done according to published procedures[10]. The purified covalently closed DNA samples were used within a few days. Fifty per cent of the DNA molecules of a ^{3}H-labelled sample with a specific activity of 2×10^5 c.p.m. μg^{-1} for example, were spontaneously converted to the nicked form by radiolysis in about 2 weeks. The membrane filter assay for repressor–operator complexes (RO) was used to titrate the repressor solution[6] as well as to measure the kinetic constants[7,8] of the repressor–operator interaction. The binding buffer used for all assays contained 0.01 M Tris buffer, *p*H 7.4, 0.01 M MgCl$_2$, 0.01 M KCl, 0.0001 M EDTA, 0.0001 M dithiothreitol, 5% dimethylsulphoxide and 25 μg bovine serum albumin per ml, which had been heated at 60° C for 30 min in 0.01 M Tris, *p*H 9, and then neturalised. The filtering buffer had the same composition as the binding buffer but without the bovine serum albumin and dimethylsulphoxide. The purified repressor and the bovine serum albumin were shown to contain negligible amounts of nuclease activity. Concentrations of DNA of from 0.6 to 3×10^{-12} M operator were used in dissociation kinetic experiments, depending on the specific activity of the DNA preparation. The halflife of repressor–operator complexes is, however, independent of concentration[7]. A fourfold excess of repressor over operator was added to the DNA in the binding buffer and the repressor–operator complexes were allowed to form during a 30-min incubation at room temperature. The dissociation reaction was initiated by addition of unlabelled operator DNA (linear λφ80 d*lac* DNA) in a 100-fold excess over the labelled operator DNA concentration, and three 0.5 ml samples were filtered on HA Millipore filters, at appropriate times. The filters were washed once with 0.5 ml of filtering buffer, dried, and counted in a scintillation counter. The values obtained for the c.p.m. retained on triplicate filters were averaged. A background value of the amount of radioactivity retained on the filters in the presence of 10^{-3} M isopropyl-β-D-thiogalactoside, an inducer of the *lac* operon, was measured in each experiment and subtracted. The data are presented as the fraction of the amount of repressor–operator complex (RO) present before the addition of the unlabelled operator DNA. The data shown represent averaged values obtained in several independent experiments for each DNA. The initial slopes of the RO complex decay curves were calculated by least-square fitting of the data. ○, $\sigma = -0.001$, average of four experiments $t_{1/2} = 22$ min; ■, $\sigma = -0.017$, average of four experiments $t_{1/2} = 35$ min; ▲, $\sigma = -0.035$, average of five experiments $t_{1/2} = 105$ min.

The rates of dissociation (k_b) and association (k_a) have been measured for the binding of the *lac* repressor to five covalently closed circular λp*lac* DNA samples with superhelical densities σ (defined as the number of superhelical turns per ten base pairs[3] and based on an unwinding angle of 12° for ethidium) −0.001, −0.017, −0.028, −0.035 and −0.049 (or 5, 80, 160 and 230 negative superhelical turns respectively) under the binding conditions. Figure 2 illustrates the results for the rate of dissociation of the repressor from operator DNAs with −5, −80 and −160 superhelical turns. The negative superhelical turns of the DNA clearly stabilise the repressor–operator complex. The halflife of the complex is 22 min for the essentially untwisted DNA (−5 superhelical turns) and 105 min for the DNA with −160 superhelical turns. Results obtained for all DNA samples are summarised in Table 1.

Measurements of k_a are somewhat less accurate. Representative data are illustrated in Fig. 3. A small increase is observed with increasing negative superhelicity. Results obtained for all DNA samples are presented in Table 1.

Table 1 Affinity of the *lac* repressor for operator DNAs with various degrees of superhelicity

σ	$t_{1/2}$ (min)	$k_b \times 10^4$ (s^{-1})	$k_a \times 10^9$ (mol^{-1}s^{-1})	$K_a \times 10^{-13}$ (mol^{-1})	K_a^σ/K_a	θ (degrees)
Linear	25	4.7 ± 0.3	2.4 ± 0.5	0.5		
−0.001	22	5.4 ± 0.2	3.4 ± 0.6	0.6		
−0.017	35	3.3 ± 0.2	5.7 ± 0.5	1.7	2.8	43
−0.028	65	1.8 ± 0.3	4.4 ± 1.1	2.5	3.9	35
−0.035	105	1.1 ± 0.3	9.2 ± 1.0	8.5	13.6	53
−0.049	78	1.5 ± 0.1	7.5 ± 1.0	5.1	8.7	31

The halflives ($t_{1/2}$) of the different repressor–operator complexes were measured as described in the legend to Fig. 2. The rate constant of dissociation (k_b) is related to $t_{1/2}$ by the relationship $k_b = 1.17 \times 10^{-2}/t_{1/2}$, where $t_{1/2}$ is expressed in min. The rate constants of association (k_a) of the repressor to the different operator DNAs were measured as described in the legend to Fig. 3. The values shown represent the mean value of N experiments and the errors were calculated as $E = 1/N\sqrt{\Sigma (X-\bar{X})^2}$ where $X-\bar{X}$ is the deviation from the mean for each experiment. In general $N = 4$ except in the cases of the measurement of k_b with the DNA of $\sigma = -0.035$ ($N = 5$) and of k_a with the DNA of $\sigma = -0.035$ ($N = 5$) and of k_a with the DNAs of $\sigma = -0.017$ ($N = 2$), $\sigma = -0.035$ and $\sigma = -0.049$ ($N = 3$). The uncoiling angle of θ of the DNA helix due to the binding of a *lac* repressor is calculated from the equation[1]

$$K^\sigma_a/K_a = \exp(-1/RT\ (dG_\tau/d\tau)\ (\theta/360)),$$

where K^σ_a and K_a are the equilibrium constants for the binding of the repressor to DNAs with superhelical densities σ and *o* respectively, and $dG_\tau/d\tau$ is the free energy change per unit change in τ, the number of superhelical turns. The recent unpublished results of Hsieh and Wang, $dG_\tau/d\tau = 505\,RT\sigma$, were used in the calculation of θ. Previously Bauer and Vinograd[1] gave $dG_\tau/d\tau = RT\,(844\,\sigma - 2640\,\sigma^2)$. If the results of Bauer and Vinograd are used, the calculated θ values are lowered by approximately a factor of 1.5.

The affinity of the repressor for the operator, $K_a = k_a/k_b$, increases with increasing negative superhelicity up to a factor of approximately 14 for the DNA with −160 superhelical turns. Its affinity for the highly superhelical DNA with −230 turns is somewhat lower than for the DNA with −160 turns. The reason for this decrease is not clear. It has been observed[12] that for highly twisted PM2 DNAs with a negative superhelical density greater than ~0.045, the template activity of the DNA for transcription by *E. coli* RNA polymerase also decreases with increasing negative superhelicity. It is plausible that for such highly twisted DNAs, in addition to the free energy of superhelix formation[3], other factors such as steric hindrance, change of hydration of the DNA helix, and structural distortion of the helix[13] are also affecting the interactions between the DNA and other macromolecules.

From the dependence of the equilibrium constant K_a on σ, the unwinding angle θ of the *lac* operator due to the binding of a repressor can be calculated from the free energy of superhelix formation[4] The values are tabulated in the last column of Table 1 On the binding of a *lac* repressor, the operator unwinds by about 40°, or approximately one tenth of a helical turn This small unwinding cannot be reconciled with the prediction of the Gierer model for the operator structure shown in Fig 1*b*, unless the operator exists as the hairpinned structure in the absence as well as in the presence of repressor The melting temperature of the operator fragment[2] indicates, however, that it is highly unlikely that the operator assumes the hairpinned structure in the absence of repressor

We also tried to measure the unwinding of the *lac* operator by the method of Saucier and Wang[14] No definitive conclusion was obtained by this technique, which is not unexpected since the estimated value for θ is approximately the limit of detection of this technique

Maniatis and Ptashne[5] have shown, by measuring the amounts of λ repressor bound at equilibrium to linear and superhelical λ DNAs, that virtually no unwinding of the λ operator occurs on binding of the λ repressor The observed difference in affinity for the two DNAs was less than a factor of two For superhelical λ DNA isolated from infected cells, which was used in their measurements, σ is expected to be in the range of 0 026 to 0 031 (ref 15) If λ repressor unwinds the λ operator to the same extent as in the case of the *lac* system, the difference in affinities for the superhelical and linear λ DNA samples used would be approximately a factor of 4 (Table 1) It therefore appears that λ repressor does not unwind the λ operator as much as the *lac* repressor unwinds its operator, if at all

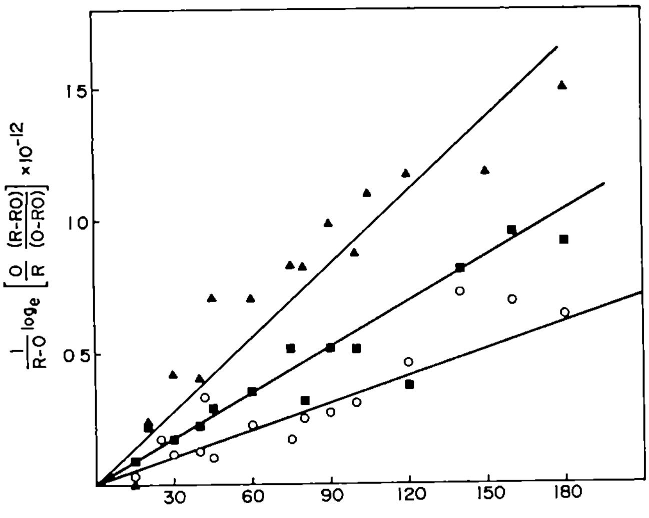

Fig 3 Rates of association of the repressor to operator DNAs with various degrees of superhelicity The conditions of the binding assay are the same as described in the legend of Fig 2 except that the concentrations of the operator DNA varied from 0 4 to 1 × 10^{-12} M and that only a twofold excess of repressor was used The total operator and repressor concentrations were determined precisely and the concentration of the repressor operator complex (RO) was calculated using a conversion factor estimated by filtering a known amount of RO complex under the same experimental conditions[7] The reaction was initiated by mixing the repressor and the operator DNA and the association was stopped at appropriate times by transferring 1 5 ml samples into 100 μl of a mixture containing 4 μg of unlabelled λΦ80 d*lac* DNA and 10^{-2} M *o*-nitrophenyl-β-D-fucoside The presence of about 100-fold excess of unlabelled operator DNA prevents the formation of new labelled RO complexes while the anti-inducer, *o*-nitrophenyl-β-D-fucoside, stabilises the existing labelled RO complexes[11] Three 0 5-ml samples of that mixture were filtered, washed and counted and the triplicate values were averaged, as described earlier The background value of the amount of radioactivity retained on the filters in the presence of 10^{-3}M isoprophyl-β-D-thiogalactoside was subtracted from each time point The data were plotted according to the rate equation for a bimolecular reaction[7], with R and O denoting the total repressor and operator concentrations respectively The rate constant of association, k_a, was obtained from the slope of the least-square line ○, $\sigma = -0\ 001$, average of four experiments, $k_a = 3\ 4 \times 10^9$ mol^{-1} s^{-1}, ■, $\sigma = -0\ 017$, average of two experiments, $k_a = 5\ 7 \times 10^9$ mol^{-1} s^{-1}, ▲, $\sigma = -0\ 035$, average of three experiments, $k_a = 9\ 2 \times 10^9$ mol^{-1} s^{-1}

In the calculation of θ from the free energy of superhelix formation, it has been implicitly assumed that the intercalative dye ethidium unwinds the DNA helix by 12° Recent results of one of us (J C W) indicate that the unwinding angle for ethidium is likely to be significantly larger than 12° with 26° being the best estimate This would lead to a proportionately larger value for θ of about 90° Since the size of the *lac* operator is about 30 base pairs, an unwinding of 40° to 90° suggests a significant change of the operator structure on the binding of the *lac* repressor

Thus the conclusions of our unwinding measurements are twofold On one hand, our results suggest that the recognition of the operator by the *lac* repressor is primarily through interactions with groups of the bases in the groves Recognition either by the Gierer model or by the disruption of a segment of the base pairs, thus exposing a sufficient number of bases, is inconsistent with our results On the other hand, the specific interaction between the operator and the repressor involves a structural change of the operator, as evidenced by the unwinding measured This structural change is likely to be important in recognition

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Subunit structure of chromatin

IN the past, the structure of chromatin has remained obscure despite the efforts of many laboratories and seemed far from a simple solution[1] It has been known only that chromatin contains a repeating substructure[2 3] Recently, however, Kornberg proposed[4] that chromatin structure is based on a repeating subunit of 200 base pairs of DNA and two of each of the histones (with the exception of F1 which occurs only once per subunit). Furthermore, he suggested that in chromatin these subunits form a flexibly jointed chain.

Here I present evidence in favour of this model. It is shown that 85% of chromatin can be converted to a subunit by digestion with micrococcal nuclease. This subunit contains about 205 base pairs of DNA and occurs as a discrete complex in solution. As well as demonstrating the existence of a chromatin subunit, the methods described here provide a means of isolating the subunit on a preparative scale and represent a test for the native state of chromatin.

Previous work has shown that a considerable amount of the DNA in rat liver nuclei is cleaved to integral multiples of a unit length upon digestion by an endogenous Ca^{2+} and Mg^{2+}-dependent endonuclease[3]. I have confirmed this result using the enzyme purified from rat liver nuclei, and demonstrated that the same DNA fragments are obtained after digestion with micrococcal nuclease (Fig 1). A similar result is obtained with DNase I although the pattern is obscured by a very high background. Whereas both the rat liver enzyme and micrococcal nuclease require Ca^{2+}, DNase I requires only Mg^{2+}. Thus, the occurrence of regularly spaced cleavage sites is not dependent on the presence of a specific divalent cation.

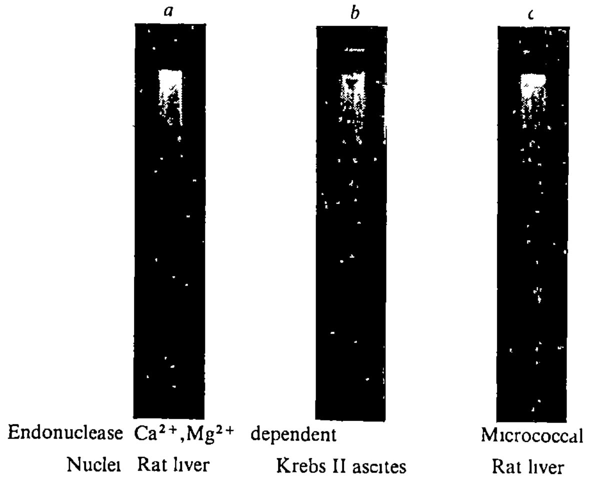

Fig 1 Polyacrylamide gel analysis of DNA digested *in situ* with micrococcal nuclease or Ca^{2+}, Mg^{2+} dependent endonuclease. *a*, Rat liver nuclei containing the Ca^{2+}, Mg^{2+} dependent endonuclease were incubated at 1.5×10^8 nuclei ml^{-1} as described[3] in the presence of 10 mM $MgCl_2$ and 1 mM $CaCl_2$ for 60 min at 37° C. *b*, Krebs II ascites nuclei which lack the Ca^{2+}, Mg^{2+} dependent endonuclease[6] were incubated as in (*a*) in the presence of 0.5 units ml^{-1} of purified Ca^{2+}, Mg^{2+} dependent endonuclease (M. N., to be published). *c*, Incubation of rat liver nuclei with 300 units ml^{-1} of micrococcal nuclease (Worthington Biochemical Co.) in 1 mM $CaCl_2$ for 15 s at 37° C. After incubation the DNA was extracted, precipitated and analysed on 2.5% acrylamide gels essentially as described[3,6].

Having established that readily available nucleases produce the same digestion pattern as the Ca^{2+}, Mg^{2+} dependent endonuclease, it was possible to answer many questions concerning the hypothetical chromatin subunit. For example, the proportion of chromatin based on this subunit structure could be determined. For this purpose it was necessary to determine the proportion of DNA that can be broken down into monomers (DNA of unit length associated with the hypothetical chromatin subunit of which the sizes of the larger DNA fragments [dimers, trimers, and so on] are integral multiples) or resolved multiples thereof if it is digested *in situ* with saturating amounts of the micrococcal enzyme. It is evident from Fig 2 that nearly all DNA is converted to the monomer size. In addition to the analysis of the DNA fragments on gels, the amount of TCA soluble DNA had to be taken into account in order to obtain a reliable value for the proportion of chromatin consisting of the repeating subunit. Under conditions converting all the DNA on the gel to monomers and resolved multiples of it (Fig 2*a*), 13% of the DNA was acid soluble. This shows that at least 87% of the chromatin consists of the subunit, since the background of DNA between the bands is extremely low (as judged by its evaluation between the well separated first three bands). This estimate represents only a lower limit since some of the TCA soluble DNA is probably due to partial degradation of DNA in the substructure as indicated by the finding that further digestion produced more acid soluble DNA (28% in Fig 2*c*).

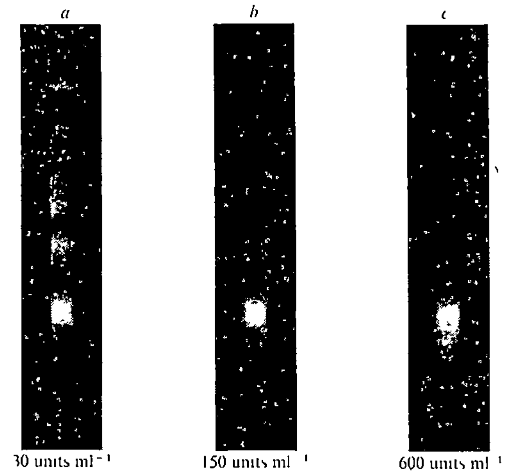

Fig 2 Titration of chromatin digestion with micrococcal nuclease. Rat liver nuclei were incubated for 6 min at 37° C in a standard reaction mixture as described for Fig 1*c* with increasing concentrations of micrococcal nuclease. *a*, 30 units ml^{-1}, *b*, 150 units ml^{-1}, and *c*, 600 units ml^{-1}. Incubation mixtures were processed and analysed as described in the legend of Fig 1.

It is evident from Fig 2 that increasing digestion with micrococcal nuclease is accompanied by the appearance of fragments smaller than the monomer which might arise by cleavage at a few sites located within the subunit. After relatively mild digestion, on the other hand, the monomer can be observed to consist of a doublet (Fig 3). Increasing digestion shifts the relative intensities of the two bands in favour of the lower band which suggests that it is produced from the upper band. Thus it is conceivable that the upper band is the true monomer while the lower band arises by cleavage at a preferred internal site of the monomer.

To clarify the relationship between the monomer doublet and the higher DNA bands an accurate determination of their size and breadth is required. This was achieved by calibration with a series of sequenced DNA fragments generously supplied by Dr J. W. Sedat. Thus the DNA of a micrococcal nuclease digest, the Hin fragments and a set of sequenced single-stranded Endo IV products of ΦX174 were run in parallel on a 99% formamide slab gel (not shown). From the sharpness of the Hin fragment bands it was apparent that no partial renaturation occurred on the gel. A logarithmic plot of the molecular weight of the Hin (P. G. N. Jeppesen, personal communication) and Endo IV (ref. 5 and unpublished results of J. W. Sedat and F. Sanger) products (the lengths of the Hin fragments had been determined by EM and did not deviate from their true value by more than 10% as judged by comparison with the sequenced Endo IV products) as a function of the square root of their mobility, resulted in a smooth calibration curve which was linear in the range of 200–400 bases. From comparison with this calibration curve the following values were obtained for the monomer doublet, dimer and trimer monomer doublet=205±15 base pairs and 170±10 base pairs, dimer=405±35 base pairs, trimer=605±40 base pairs (The breadth of the bands reflects true heterogeneity in size rather than diffusion in the gel—see also ref 6) The sizes of the monomer bands are consistent with an estimate of the average molecular weight of monomer DNA from sedimentation analysis on sucrose gradients[6]

The measurements clearly show that no multiples of the lower monomer band are found and strongly suggest that both dimer and trimer correspond to twice and three times the length of the upper monomer band rather than a linear combination of both monomer bands The data, therefore, favour a model in which the monomer doublet consists of the real monomer (205 base pairs) and a degradation product of it (170 base pairs) produced by cleavage at a site located approximately 20% within the monomer No 'monomers', however, are observed arising from cleavage at this site in the dimer, that is, the monomer has never been observed as a triplet (with a third band of 240 base pairs) A possible explanation would be that the internal cleavage site only becomes accessible in the free subunit

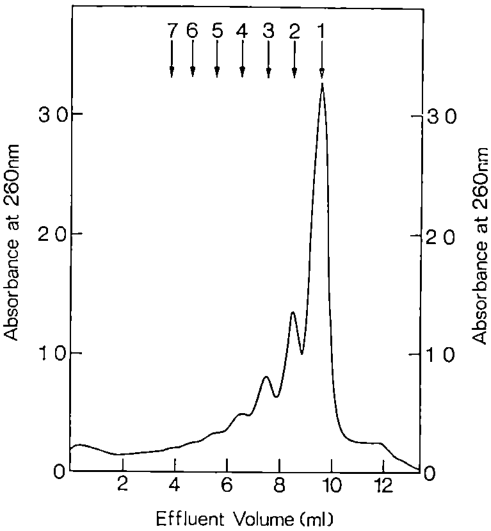

Fig 4 Sucrose gradient analysis of digested chromatin Digested chromatin was prepared from rat liver nuclei as described for Fig 3 except that incubation was for 2 min rather than 15 s The chromatin supernatant (5 5 A_{260} units) was layered on a 11 5-ml isokinetic sucrose gradient (D Calhoun, M N and H Noll, in preparation) with $c_t=5\%$, $c_r=28\ 8\%$, and $V_m=55\ 5$ ml in 0 2 mM EDTA, *p*H 7 and centrifuged for 12 75 h at 28,000 r p m and 4° C in a Beckman SW40 rotor The gradient was monitored for absorbancy by passing the effluent from the bottom of the tube through a turbulence free flow cell (Molecular Instruments Co , PO Box 1652, Evanston, Illinois 60201, USA)

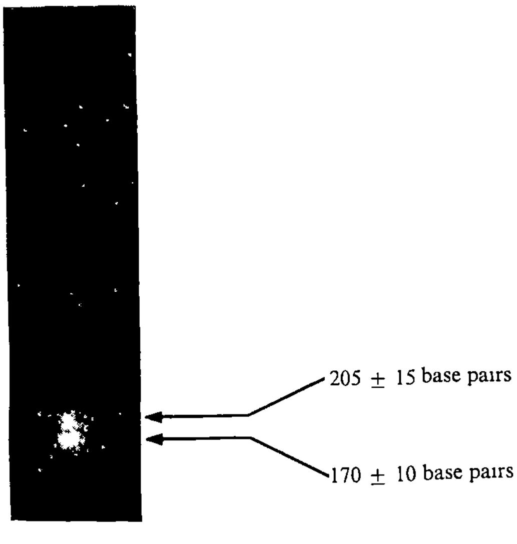

Fig 3 Monomer doublet after 'mild' micrococcal nuclease digestion After incubation of rat liver nuclei with micrococcal nuclease (15 s at 37° C, 150 units ml^{-1}) the nuclei were pelleted at low speed, resuspended in 0 2 mM EDTA, and homogenised with a Waring blender The homogenate was centrifuged for 30 min at 12,000*g* and the supernatant containing the digested chromatin was processed and analysed as described for Fig 1

The results presented so far concern primarily the composition of the chromatin subunit Evidence that the subunit occurs as a discrete complex in solution comes from sucrose gradient analysis of chromatin previously digested with micrococcal nuclease As demonstrated in Fig 4 a regular series of seven resolved peaks is observed which have been shown to contain DNA of the size of the monomer (first peak), dimer (second peak), trimer (third peak) and so on Thus histone–histone interaction between adjacent subunits is not sufficient to hold them together Since the sucrose gradient is isokinetic (for a particle density of 1 5 g cm^{-3}) the S-values are proportional to the distance migrated in the gradient[7] By calibration with 30S ribosomal subunits of *Escherichia coli*[8] it was shown that the chromatin subunits sediment at 11 2±0 4S and the chromatin dimers at 15 9±0 5S These values have been confirmed by sedimentation velocity analysis in an analytical ultracentrifuge The first two peak fractions when fixed with formaldehyde band both as a homogenous peak with a buoyant density of 1 45 g cm^{-3} in a CsCl gradient, exhibit a protein to DNA ratio of approximately 1 3 and contain in addition to all five major histones some non-histone proteins Van Holde and coworkers have reported a 12S product of micrococcal nuclease digested chromatin[9] The 12S material differs from the 11S subunit reported here in size of DNA (about 100 rather than 205 base pairs) and protein to DNA ratio (1 5 rather than 1 3) The 12S material may arise from the 11S subunit through over-digestion (ref 4, footnote 8)

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Protein synthesis and the release of the replicated chromosome from the cell membrane

In *Escherichia coli*, chromosome replication must be followed by a short period of protein synthesis for subsequent division of the cell[1] The protein synthesised during this period has been termed 'termination protein'[1,2] We have set out to test the hypothesis[2] that this post-replication protein synthesis is required to release the chromosomal DNA from its attachment to the cell membrane and that this detachment is necessary for subsequent cell division The results of the experiments reported here provide support for this model

A recently-described technique permits the isolation of the chromosome of *E coli* as a highly folded condensed structure complexed with small amounts of RNA and protein[3] Both the RNA and the protein (which is largely RNA polymerase) are required for maintaining the chromosome or 'nucleoid' in its condensed state[4,5] It has been hypothesised that this folded form is the normal state of the chromosome within the intact cell When a modified extraction procedure is used, the folded chromosome can be isolated attached to fragments of the cell membrane[4,6] According to Worcel and Burgi[6], the chromosome becomes detached from the membrane when replication is completed in the absence of a required amino acid, but becomes re-attached some time after the addition of the amino acid They therefore conclude that the chromosome is attached to the membrane during replication and is released after the completion of replication We describe here experiments which confirm these findings and also show that protein synthesis is required for the release of the replicated chromosome from the cell membrane

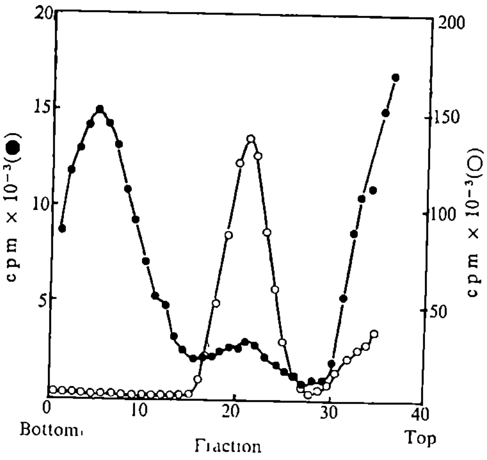

Fig 1 Sucrose gradient profiles of cell lysates prepared at 0° C and at 25° C ^{3}H-thymidine (0 5 μCi ml^{-1}, 90 4 mCi mg^{-1}) was added to an exponential culture of *E coli* Km7T$^-$ growing at 37° C in 007-glucose minimal medium[1] supplemented with threonine (50 μg ml^{-1}), leucine (50 μg ml^{-1}), thiamine (10 μg ml^{-1}) and thymine (10 μg ml^{-1}) Growth was continued for 60 min and then the cells were rapidly harvested and lysed essentially as described by Stonington and Pettijohn[3] The cells were harvested by centrifugation and the pellet (2–3 × 10^9 cells) resuspended at 0° C in 0 4 ml of a solution containing 0 01 M Tris (*p*H 8 1), 0 01 M sodium azide, 0 1 M sodium chloride, 20% sucrose (w/v) and 0 01% diethyl-pyrocarbonate (added separately for each experiment) Aliquots (0 1 ml) of a 30 mg ml^{-1} solution of lysozyme in 0 12 M Tris (*p*H 8 1) and 0 05 M EDTA were immediately added and 30 s later 0 5 ml of a lysis solution containing 1% Brij-58, 0 4% deoxycholate, 2 M sodium chloride, 0 01 M EDTA (*p*H 8 1) was added Lysis was allowed to continue at 0° C or 25° C for 10 min The lysate was spun at 4,000*g* at 0° C for 5 min and the supernatant layered on to a 5 ml 10–30% (w/v) sucrose gradient containing 0 01 M Tris (*p*H 8 1), 1 M sodium chloride, 0 001 M EDTA and 0 001 M 2-mercaptoethanol The gradients were spun in a Beckman SW50 1 rotor for 20 min at 17,000 r p m and at 2–4° C Fractions were collected on Whatman 3MM filters and the filters washed in 10% TCA (2×) and acetone, dried and counted in a toluene based scintillation fluid ●, Cells lysed at 0° C, ○, cells lysed at 25° C

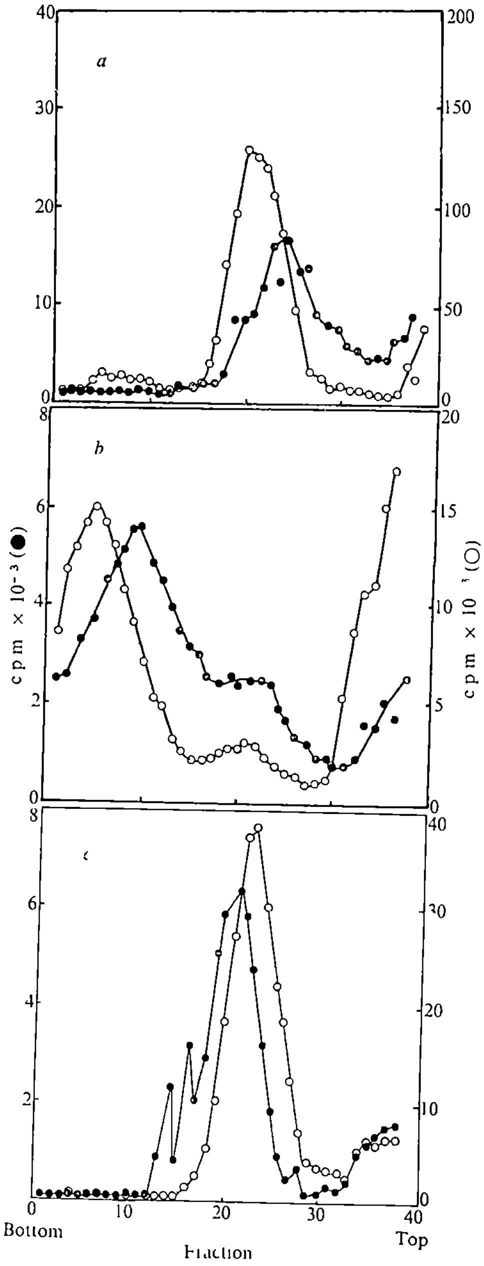

Fig 2 Detachment of nucleoids from the membrane ^{3}H-thymidine (0 5 μCi ml^{-1}, 90 4 mCi mg^{-1}) was added to an exponentially growing culture of *E coli* Km7T$^-$ in supplemented minimal medium (see Fig 1) After 60 min, cells were treated in one of three ways *a*, Cells were incubated for 60 min with rifampicin (500 μg ml^{-1}) and then washed on a membrane filter with prewarmed medium[1], after which they were resuspended in fresh medium and nucleoids prepared at 4° C 10 min later *b*, Cells were incubated in rifampicin and washed as in (*a*), but resuspended in medium containing chloramphenicol (160 μg ml^{-1}) before preparation of nucleoids at 0° C 10 min later *c*, Cells were incubated for 50 min with chloramphenicol (160 μg ml^{-1}) before preparation of nucleoids at 25° C ●, Nucleoids prepared from treated cells at 0° C (*a* and *b*) or 25° C (*c*) ○—○, Nucleoids prepared from exponentially growing cells at 0° C (*b*) or 25° C (*a* and *c*)

E coli Km7T$^-$ was used in these experiments In this strain RNA synthesis can be rapidly inhibited by the addition of rifampicin and then restarted by its removal[7] In the presence of rifampicin (as in the absence of required amino acids) chromosome replication completes but is not reinitiated[1] It was found necessary to use rifampicin rather than amino acid starvation because the latter allowed considerable turnover of protein[8–10] This turnover was found to be sufficient to allow the synthesis of termination protein (N C J, unpublished results)

Nucleoids were isolated from the cells by the procedure of Stonington and Pettijohn[3] We found the sedimentation of these bodies on sucrose gradients to be very similar to that described by other workers[4 6] When exponentially growing cells were lysed at 25° C all the DNA (prelabelled with ^{3}H-thymidine) was found in a broad, slowly sedimenting peak characteristic of membrane-free nucleoids (Fig 1) When the cells were lysed at 0° C, however, the DNA was found to be mainly in a fast sedimenting peak characteristic of membrane-bound nucleoids (Fig 1) In the cells lysed at 0° C, a minor fraction of the DNA sedimented at the rate expected for membrane-free nucleoids (as found also by others[6]) These may represent the chromosomes extracted from the cells which had completed their rounds of chromosome replication, as the proportion observed (about 17%) is close to the expected proportion of DNA which is in non-replicating chromosomes in an asynchronous exponential population with the observed generation time (about 60 min)[11]

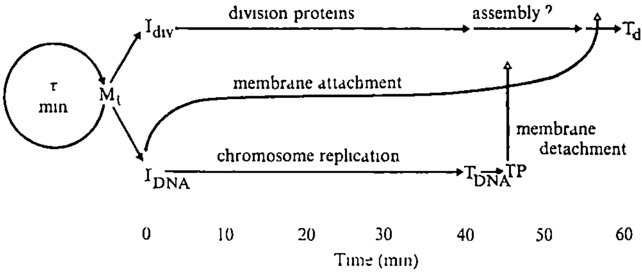

Fig 3 Model of the cell cycle in *E coli* (modified from Jones and Donachie[1], Fig 6) Doubling of the initiation mass (M_i[13]) takes place every mass doubling time (τ min) At each doubling chromosome replication (I_{DNA}) and synthesis of a set of division proteins (I_{DIV}) are initiated approximately simultaneously Completion of the synthesis of division proteins takes place at about 40 min and this in turn initiates a sequence of events (IA) which takes 20 min to complete, does not require the synthesis of DNA, RNA or protein and which leads to cell division Initiation of chromosome replication requires the attachment to a specific membrane site and this attachment prevents the completion of a terminal stage in the division sequence Termination of DNA replication (T_{DNA}) after 40 min however, induces the synthesis of termination protein (TP) which is required for the release of the replicated chromosomes from the membrane and, consequently, for eventual cell division

In agreement with other workers, it was found that folded chromosomes could not be isolated from cells treated with rifampicin[4] This is in accord with the idea that continuing RNA synthesis is necessary to maintain the nucleoid in its folded form The chromosomes are recovered in the folded form within 10 min or less of removing the rifampicin, however, even in the presence of chloramphenicol, and so only a small amount of new RNA synthesis and no new protein synthesis is needed for refolding of the chromosome

This enabled us to use the following procedure to test whether protein synthesis is needed for the dissociation of the chromosome from the membrane An exponential culture was treated with rifampicin for 60 min to allow completion of all rounds of chromosome replication The rifampicin was removed and RNA synthesis resumed for 10 min (to allow refolding of the chromosome) in the presence or absence of chloramphenicol Nucleoids were then prepared from these cells at 0° C If protein synthesis is necessary for detachment, then this detachment would be prevented by the chloramphenicol and so the nucleoids would remain membrane bound The results in Fig 2 show that this is indeed the case The nucleoids prepared from the cells that were allowed to resume RNA synthesis in the absence of chloramphenicol, sedimented at a position characteristic of membrane-free nucleoids (Fig 2*a*) When chloramphenicol was present, however, the nucleoids prepared were found to be membrane bound (Fig 2*b*) Fig 2*c* shows the sedimentation coefficients of nucleoids prepared from untreated exponentially growing cells and cells treated with chloramphenicol The results show that the chloramphenicol does cause a small increase in the sedimentation coefficient of the nucleoid[12] but this increase is not significant enough to account for the above results

The sedimentation peaks of both the membrane-bound and the membrane-free nucleoids were slightly displaced towards the top of the gradient by comparison with the corresponding membrane-bound and membrane-free nucleoids prepared from exponential cells This would be expected if chromosome replication were completed during the rifampicin treatment because the nucleoids should each consist of a single unreplicated chromosome while those from exponential populations should consist of chromosomes in all stages of replication and contain 1–2 chromosome equivalents of DNA[6]

A small fraction of the nucleoids from the cells to which chloramphenicol was added after the removal of rifampicin (Fig 2*b*) was membrane free This proportion was similar to the proportion of membrane-free nucleoids prepared at 0° C from exponential cells (Fig 1) The cells from which these were obtained had presumably completed replication before the addition of the rifampicin

The results reported here therefore confirm those of Worcel and Burgi[6] but in addition show that a short period of protein synthesis (10 min or less) after the completion of DNA replication is required for the release of the folded chromosome from the membrane We have shown previously that termination protein synthesis at this same time is a necessary prerequisite for subsequent division[1] It is therefore tempting to speculate that the role of termination protein is to release replicated chromosomes from their sites of membrane attachment and that therefore it is the attachment of the chromosome to a membrane site (at initiation of DNA replication) which sets up a block to cell division The subsequent release of this attachment, at termination of replication, would then abolish the block to division

Figure 3 represents our present model for the control of cell division in *E coli*, modified from that given earlier[1] to include the suggested role of termination protein

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Sodium pump stoichiometry in *Aplysia* neurones from simultaneous current and tracer measurements

THERE is growing evidence for the involvement in neuronal performance of hyperpolarising current resulting from the activities of the membrane 'sodium pump'[1] Studies of the stoichiometry of metabolically fuelled ionic movements[1–6] have consistently shown that more Na is extruded than K accumulated But while the electrical effect of such an imbalance is of immediate significance to the ongoing activity of a neurone, we are aware of only one study in which the electrogenicity of sodium pump activity in a neurone has been estimated[7] Here we make such an estimate by comparing simultaneous measurements from a single neurone somata of tracer Na efflux and the transmembrane current attributable to that efflux The results show that at least half of the actively extruded Na is electrogenic

The experiments were carried out on the giant cell, 500–700 μm in diameter of the abdominal or pleural ganglions of *Aplysia californica*[8], iontophoretically injected with ^{24}Na The experimental arrangements are presented diagrammatically in Fig 1

Figure 2 shows the variations with time of the membrane potential [*E*m], pump current (*I*), intracellular Na activity ([Na]$_i$) and ^{24}Na efflux (φ) following two steps of constant and sustained injection of ^{24}Na As soon as the ^{24}Na injection was started by passing a 50 nA current between the two injection electrodes ($t=0$ in Fig 2) hyperpolarisation developed (lower curve in Fig 2) Concomitantly, a positive value of pump current was recorded After a small delay, partly due to dead space in the washout system, the efflux also increased Internal Na increased and, together with the other parameters recorded, reached a new equilibrium level after 45 min constant injection Observations such as these show that in these experiments a steady state was reached, in which Na efflux matched the injection rate When the injection was doubled, all parameters again rose and reached higher equilibrium values

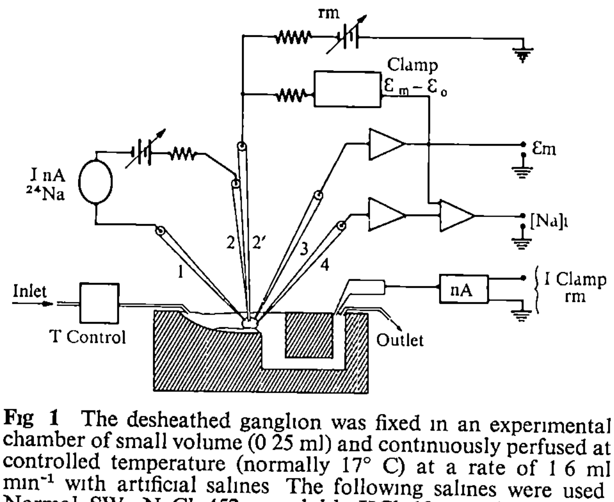

Fig 1 The desheathed ganglion was fixed in an experimental chamber of small volume (0 25 ml) and continuously perfused at controlled temperature (normally 17° C) at a rate of 1 6 ml min^{-1} with artificial salines The following salines were used Normal SW, NaCl, 452 mmol l^{-1}, KCl, 10 mmol l^{-1}, CaCl$_2$, 11 mmol l^{-1}, MgCl$_2$, 49 mmol l^{-1}, NaHCO$_3$, 6 mmol l^{-1}, K-free SW, same as normal SW minus KCl, Na-free (Tris) SW, Tris 513 mmol l^{-1}, KCl, 10 mmol l^{-1}, CaCl$_2$ 11 mmol l^{-1}, MgCl$_2$ 49 mmol l^{-1}, Na-free (Mg mannitol) SW, MgCl$_2$, 283 mmol l^{-1}, CaCl$_2$, 11 mmol l^{-1}, KHCO$_3$ 10 mmol l^{-1}, mannitol, 234 mmol l^{-1} Controlled amounts of Na were injected into the cells between electrode 1 (2 M ^{24}NaCl solution, 15 mCi mmol^{-1}) and electrode 2 (KCl) Every 3 min, a slow time-constant voltage clamp device reset the membrane potential, $\mathcal{E}_m$ (measured with a separate electrode 3), to the initial value, $\mathcal{E}_0$, for about 30 s by injecting a current across the membrane (electrode 2′) This current was considered equivalent to the additional pump current in response to the Na injection[7] Effluents were collected for 3 min periods and the ^{24}Na radioactivity counted In some experiments the intracellular Na activity was measured with an Na-sensitive microelectrode (electrode 4) according to Thomas' procedure[9]

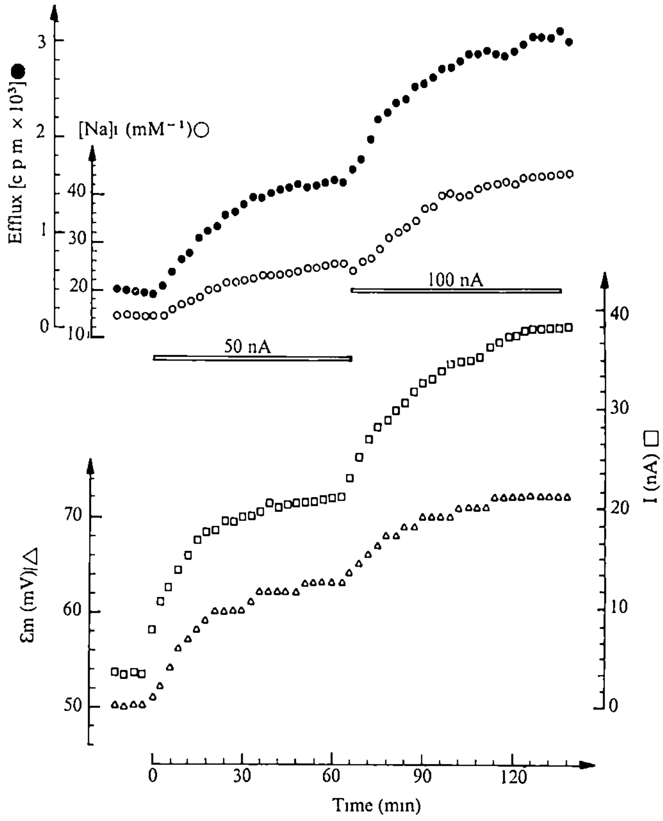

Fig 2 Time course of membrane potential (△), clamp current (□), intracellular Na activity (○) and efflux of ^{24}Na (●) from a giant cell following two steps of constant ^{24}Na injection Steady values are reached within 45 min of beginning a constant current ^{24}Na injection

We have used strophanthidin to define the fraction of the total ^{24}Na efflux which represents active extrusion The data are summarised in Table 1 When strophanthidin (10^{-3} M^{-1}) was added to the perfusing saline, ^{24}Na efflux was reduced to about half, whether the saline was natural seawater (NSW) or a Na-free seawater (SW) Contrary to the effects of ionic concentration changes, the effects of strophanthidin were irreversible

We have further characterised the components of ^{24}Na efflux by testing the effects of K-free salines, cyanide and cold The omission of K from an otherwise normal artificial seawater decreased total ^{24}Na efflux by about 40%, but only by 15% in Na-free SW After strophanthidin, the removal of external K had no effect on the remaining efflux These results indicate that external K ions activate the strophanthidin-sensitive component of Na efflux[4] Cyanide (2×10^{-3} M), perfused for as long as 1 h reduced ^{24}Na efflux no more than did strophanthidin, or if applied after strophanthidin, had no effect

Finally, when perfusing saline was cooled to 3° C, efflux was reduced by 80% This extreme drop in temperature

Table 1 Percentage reduction of ^{24}Na efflux

	K free	Strophanthidin (10^{-3} M)	CN^- (2 10^{-3} M)	Cold (3° C)
Na SW	43±3 (14)	56±4 (9)	42±5 (3)	83±3 (7)
Na-free SW	15±2 (9)	53±3 (10)	30±7 (2)	81±5 (6)

Fractional inhibition of ^{24}Na efflux (mean ± s e m) expressed as (φ10K − φ*x*)/φ10K × 100, where φ10K is the number of counts before changing the conditions and φ*x* the number after that change, in NSW or in Na-free SW, changes to K-free SW, strophanthidin (10^{-3} M l^{-1}), CN^- (2 10^{-3} M l^{-1}), or cold Na was substituted by Tris or Mg mannitol[9] Number of experiments in parentheses

therefore not only decreased the strophanthidin-sensitive component of ^{24}Na efflux, but also the insensitive one

Data obtained with internal Na-sensitive electrodes indicate that after 1 h in Na-free saline, internal Na reaches a very low level (less than 3 mM) After 1 h of iontophoretic Na injection with currents of 50–100 nA, intracellular Na activities of cells immersed in Na-free SW are steady and close to those observed in NSW, 20–30 mM Since no entry of 'cold' Na from the bath takes place, we assume the specific intracellular ^{24}Na activity to be very much the same as that of the solution in the injecting electrode In these conditions, the calculation of the electrogenicity of the active transport system of Na becomes possible when the strophanthidin-sensitive clamp current (that is, the amount of transferred charge) is compared with the strophanthidin-sensitive ^{24}Na efflux (that is, the total amount of Na activity extruded) It is worth emphasising that intracellular Na levels of our injected cells in Na-free SW are similar to those of uninjected cells in NSW, our analysis thus applied to Na pumping in a 'normal' range of intracellular Na

When in Na-free (Tris) SW (Fig 3) the efflux and clamp current reached a plateau Introduction of strophanthidin produced a rapid, sharp reduction of both ^{24}Na efflux and clamp current to new plateau levels The differences between the plateau values were used as measures of pump electrogenic current, *I*, and of active Na efflux, φ In this particular experiment, the ratio *I* φ had a value of 0 60 Table 2 presents data from similar experiments on six cells Values of the electrogenic ratio vary from 0 40 to 0 65, and are clearly not related to the manner in which substitution for Na in the saline was accomplished It should be emphasised that this electrogenic ratio does not depend on the degree of active transport inhibition by strophanthidin since it results from the comparison between the decreases in ^{24}Na efflux and current observed on one and the same cell The mean figure of 0 56 taken at face value indicated that at least half of the Na actively extruded from the *Aplysia* giant neurone carries a net positive charge

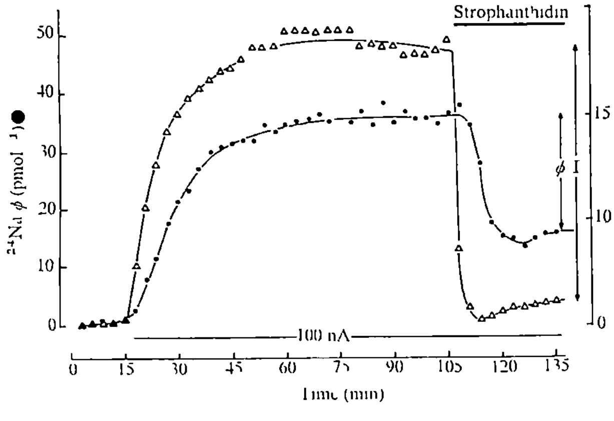

Fig 3 Efflux and pump current during constant ^{24}Na injection of a giant cell equilibrated for 1 h and perfused with Na-free (Tris) SW effect of strophanthidin (10^{-3} M) ^{24}Na injection (100 nA) was started about 15 min after beginning effluent collections and maintained from then on ●, Mean flux for each 3 min collection, △ clamp current applied for 30 s at the end of each collection period *I* and φ correspond to the strophanthidin-sensitive fraction of efflux and clamp current tabulated in Table 2

The electrogenicity and its inhibition, together with ^{24}Na efflux, by removal of external K or addition of strophanthidin, is consistent with a pump model in which extrusion of two Na^+ is balanced by the uptake of one K^+ This 2 Na 1 K ratio differs from the mostly evoked 3 Na 2 K figure In squid axon experiments, however, the data are consistent with such an exchange[2–4] In human blood cells, an apparent ratio 3 2 becomes nearly 2 1, if a correction is made for ouabain sensitive K–K exchange taking place in these cells[10] Our results seem to conflict more with Thomas' observations on snail giant neurones[7] He computed that only one of three or four Na^+ extruded carried net charge

Table 2 Clamp current (*I*), strophanthidin-sensitive efflux (Naφ) and the ratio *I* φ, for experiments in Na-free SW

Na substitute	pmol min^{-1} Naφ	*I*	*I* φ
Mg^{2+}, mannitol	20 8	8 6	0 41
Tris	19 8	9 9	0 50
Mg^{2+}, mannitol	17 0	11 1	0 65
Mg^{2+}, mannitol	24 0	13 2	0 55
Tris	22 0	14 0	0 64
Tris	20 7	12 3	0 60
			0 56 mean ±0 04 s e m

The discrepancy between these and our results might reflect a variation of a coupling Na K ratio under different experimental conditions[11–13] (for example, differences in electrochemical gradient) or differences in properties inherent in nerve cell membranes of different species We think that the answer to this question can be reached by further studies using our experimental approach in which ionic fluxes and their electrogenic impact can be measured simultaneously on a single neurone in different ionic and/or metabolic conditions

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Evolutionary significance of autogenous regulation

GOLDBERGER[1] has drawn attention to numerous reports relating both to prokaryote and eukaryote systems in which it has been shown that an enzyme may have, in addition to its own catalytic function, a regulatory role in determining the rate of its own synthesis He has suggested the term 'autogenous regulation' to describe such situations The term 'autoregulation' has previously been used by others[2,3] to describe this general phenomenon Autogenous regulation is, however, itself an example of a more widespread phenomenon, whereby a protein may have a regulatory function as well as a catalytic or structural role Thus for example, Slonimski and his coworkers[4,5] have suggested that in *Saccharomyces cerevisiae,* iso-2-cytochrome *c* regulates the synthesis, not of itself, but of iso-1-cytochrome *c,* and I have proposed[6] that in *Aspergillus nidulans,* nitrate reductase regulates not only its own synthesis, but also the syntheses of many catabolic enzymes

In an earlier paper[7], I speculated briefly on the possible evolutionary significance of this bifunctionality of certain proteins, suggesting that it might be a more widespread phenomenon than was at the time apparent Now that a number of other similar examples have been reported (see ref 1 for a list of references), it is worthwhile to develop these speculations further

I assume that, primitively, enzyme synthesis was unregulated For regulation to evolve, an element was required which must combine two specificities It had to be able to interact in some way with the apparatus of gene expression to ensure that the gene product was synthesised only when the environmental conditions of the cell required it It therefore also had to be able to recognise these environmental conditions The evolution *de novo* of such an element, with its two unrelated specificities, would pose problems There were already present in the cell, however, elements which had one of these functions At this point, it is simpler to consider the evolution of inducible and repressible systems separately

For inducible systems, the cell already possessed enzymes which had to recognise their substrates to function and it seems plausible that these would provide a starting point from which evolution could select the second specificity necessary for them to acquire additionally a regulatory function Although, for inducible systems, an enzyme would be an ideal candidate for evolving the function of regulating its own synthesis, it is by no means the only candidate Any other protein which interacted either with the substrate of the enzyme whose synthesis is to be regulated, or with some metabolite whose intracellular concentration was correlated with the concentration of that substrate, would also be suitable This may explain why the natural substrate is not the effector in some inducible systems Examples of this are xanthine dehydrogenase I in *Aspergillus nidulans* which is induced by its product uric acid but not by its substrate hypoxanthine[8], and the *Escherichia coli lac* system which is induced by allolactose, but not by lactose[9]

For repressible systems, there would also be a variety of possible candidates already present in the cell, which would be suitable for selection for a regulatory role These would include any protein which could bind either to the product of the pathway to be repressed, or to some metabolite whose concentration was correlated with the concentration of that product (for example, for some amino acid biosynthetic pathways, the charged amino-acyl tRNA) In several cases it has in fact been found that the first enzyme of the biosynthetic pathway, which recognises the end product or a related metabolite because it is subject to feedback inhibition, also has a regulatory role in determining the rate of synthesis of the enzymes of the pathway[1] These findings imply that the evolution of feedback inhibition may have predated repression

In both inducible and repressible systems, it is possible that selection for the modification of a catalytic protein so that it acquires additionally a regulatory function, may in some cases put demands on that protein such that it could perform neither catalysis nor regulation well In such cases, duplication of the gene specifying the protein, and subsequent divergence of the two gene copies, would allow each to be selected for one of the functions only This would result in genes whose protein products would now seem to have only a regulatory function, but it is possible that such proteins might still contain in their amino acid sequences 'fossil' evidence of their evolutionary origin

Beyrouther, *et al*[10] have determined the amino acid sequence of the *E coli lac* repressor, and have found no similarities between it and those parts of the *β*-galactosidase sequences which are known They argued that it was unlikely therefore that the two proteins had evolved from a common ancestor The *lac* repressor could have evolved, however, from some other cellular enzyme which bound allolactose

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matters arising

Two-stage transformation *in vitro*

THE evidence for malignant transformation of cells *in vitro* by the combined action of benzo(a)pyrene (initiator) and phorbol ester (promoter), presented by Lasne *et al*[1] is very striking I feel, however, that they fail to bring out the true nature of their results

The benzo(a)pyrene treatment of the primary rat fibroblasts was at the third passage, yet the dramatic increase in the percentage of transformed colonies was delayed until the thirty-fourth passage, when the incidence became 20 5% (benzo(a)pyrene alone) and 33 0% (with phorbol) By this time the 'primary' fibroblast culture was becoming senescent This was indicated by the rise in frequency of transformed colonies in the controls to 11 5% by the fortieth passage

It seems that we are not observing an increase in the rate of transformation but rather the selection of already transformed clones in a mixed population of cells The phorbol seems to increase the selective advantage of transformed cells by 50% There is no sign so far of two-stage transformation

Two-stage transformation becomes apparent only when 2-d-old rats were injected with 2×10^6 cells At the fortieth passage, although 42 5% of the cells were transformed by *in vitro* standards, they produced no tumours on injection At the same time, cells also exposed to phorbol produced tumours in every rat There has been a malignant transformation of the phorbol-treated cells which only becomes apparent on injection into animals

The 'two stages' of malignant transformation are therefore, first, the transformation in colony morphology as seen *in vitro*, which is induced by benzo(a)pyrene After 30 passages these transformed cells have a selective advantage and grow at the expense of the untransformed cells The selective advantage is slightly enhanced by phorbol Second, the transformation of stage 1 cells which is cryptic *in vitro*, but apparent on injection into young rats by the production of tumours This is induced, or perhaps merely accelerated, by phorbol

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DR LASNE REPLIES—Thank Dr Bateman for the analysis of our results in our paper I think however, that the problem is not so simple

So far as we know, the increase in the percentage of transformed colonies (11 5% by the fortieth passage) in tissue culture, does not necessarily indicate senescence On the contrary, it shows and confirms the beginning of an *in vitro* spontaneous cell transformation[1–3]

When Dr Bateman speaks about the senescent cell selection theory, he perhaps thinks of the Prehn's 'cloning selection theory'[4] In this case, we refer him to reports[5–7] that in chemical carcinogenesis, no selection of pre-existing transformed cells has happened

Concerning the action of phorbol ester, and in general the action of the other promoters, on the growth of pre-existing transformed cells, we wonder whether the author is aware of studies on carcinogensis *in vivo* Our results are interpreted in correlation with the model established *in vivo* In previously initiated animals, the promoter increases the tumour incidence, and shortens the latent period[8] *In vitro*, the phorbol ester gives similar results increasing the number of transformed colonies, and accelerating the tumour development after inoculation of the cells in animals But we have not discussed the general theory of cocarcinogenesis, which requires greater attention Our paper merely raises this question

Lastly, these results have been obtained and presented to the scientific public Each investigator may present his own interpretation We have proposed one

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The Musgrave Block-Amadeus Basin Complex

DAVIDSON[1] suggested that a plate tectonic model, involving continental collision, could explain the features observed in the Musgrave Block-Amadeus Basin area of central Australia Several significant features of the area are not accounted for in his model and these are, I believe, of sufficient importance to warrant modification of the model

In Davidson's model the Giles Complex is considered to represent part of an ophiolite thrust belt, originating in layers 2 and 3 of the oceanic lithosphere Bearing in mind the form of ophiolite sequences, however (see, for example, the discussions at the Penrose Field Conference[2]), there is no indication that rocks of the Giles Complex are in fact ophiolites, even if the absence of cherts and pillow lavas in the area is ignored There is a marked absence of olivine rich rocks, a deficiency of ultramafic rocks in general, and all parts of the Complex show some features characteristic of stratiform intrusions, such as cumulate textures, cyclic units, rhythmic layering and associated 'sedimentary type' structures[3,4] Repetition within the sequence of mafic–ultramafic units is largely a result of the igneous layering and cyclic units Some intrusions in the central part of the Complex show features characteristic of crystallisation under high pressure (that is, near the base of the crust[4]) and such features are incompatible with an oceanic ophiolite type origin Furthermore, there is evidence that the separate bodies of the Giles Complex show a depth stratification[4] which is inconsistent with Davidson's model

Several structural aspects and relationships are also inconsistent with that model The bodies of the Giles Complex were intruded into granulite facies rocks—chilled margins occur in places, and unfaulted boundaries are transgressive to the earliest recognisable folds in the granulites[4,5] Relatively minor, local low-angle faults have affected some intrusions such as Gosse Pile[6] and Kalka[4] The faulting occurred under conditions of high pressure and temperature, equivalent to granulite facies[7], and should not be confused with the much larger scale mylonitic faulting such as occurred in Woodroffe, Hinckley and Davenport The low-angle faulting was responsible for the partial dismembering of parts of the Giles Complex But even though the felsic units of Gosse Pile were removed in this way they can be related to the more felsic Kalka intrusion which occurs to the west, along the strike of the fault, and therefore the deformation is unlikely to have caused the total removal of the supposed pillow lavas and pelagic sediments

After the minor phase of high pressure and temperature thrusting there followed a period of regional deformation (F_2) involving both granulite facies rocks and the Giles Complex bodies (refs 3 and 4) This was followed by the intrusion of picrite plugs and olivine dolerites, and a third period of folding (F_3), before the deformation along the major E–W mylonite zones, such as the Hinckley Fault

The eastern Musgrave Ranges had a similar, complex structural history before faulting took place along the major Woodroffe Fault and Davenport Shear[8] That indicates that the Giles Complex had intruded granulite facies rocks and, in part, had crystallised and been deformed under conditions of high temperature and pressure and had undergone two further periods of deformation before major movement along the large thrust zones They are therefore obviously not ophiolites

The presence of the major thrust zones and the large volumes of mafic intrusives, indicates that this area has been one of significant tectonic activity Apart from the exposed thrust zones and rocks of the Giles Complex, a marked gravity high extends from the Musgrave Block to the Indian Ocean This is the Ankatell-Warri Ridge[9,10] These features all suggest a major uplift along the thrust zones in this area, such that upper mantle material has been brought near the surface There is evidence to suggest that the rocks to the north of the Woodroffe Fault are of lower metamorphic grade (amphibolite facies) than those to the south (granulite or granulite–amphibolite transition facies) and also have different tectonic histories[8]

Similarly, the Olia Gneiss containing nappe structures of Upper Proterozoic rocks in the southern Amadeus Basin (section *c–d* in Davidson's article) consists of amphibolite, migmatite and schist, and is not granulite facies terrain This leads me to suspect that areas to the north and south of the major thrust zones are not as similar as they may appear to be at first, and a preconvergence period of spreading within a single continent may not be necessary

A modified model can be proposed, in which a southern Musgrave craton collided with a northern Musgrave (?Arunta) craton along the line now marked by the thrust zones of the Musgrave Block and by the Ankatell-Warri Ridge The southern Musgrave craton was forced up along the thrusts, bringing to higher levels the rocks of the lower crustal granulite facies and the high pressure intrusives of the Giles Complex Further west it did not rise so high and higher level (lower pressure) intrusives and metamorphic rocks are exposed[11] Because of the uplift, upper mantle material would now be nearer the surface, giving rise to the gravity anomalies At a very high level the ophiolite complexes, folded sediments and lower grade (including blueschist) facies metamorphics would have been found, but considerable erosion has removed these, and the remnants may be found in the Upper Proterozoic Inindia and Winnalla Beds and in the Cambrian Mt Currie conglomerate, as suggested by Davidson

The 'Woodroffe-Ankatell Suture' may thus be regarded as a major tectonic boundary and, in the same way as the Insubric Line of Europe may mark the site of a Mesozoic collision trench (for example, the Ivrea Zone[12]), the 'Woodroffe-Ankatell Suture' may mark the site of a Proterozoic collision trench In the Australian example, however, a considerably deeper section of the boundary is exposed If this model is correct it implies that the radiometric ages of rocks to the north of the Woodroffe Fault should be younger than those to the south

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Matters Arising

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Musgrave Block

MOORE'S comments require that some modification be made in the detail of the original model[1] The degree of modification, however, is not nearly as extensive as might be implied from the criticisms he presents

His arguments are related principally to two points First, the nature of the mafic-ultramafic rocks of the Giles Complex, their origin and mode of emplacement Second, the time-space relationship between the colliding continental blocks My following comments are concerned with answering objections raised by Moore in regard to these points

The geological environment in which layered or stratiform mafic–ultramafic complexes are formed is still uncertain and it is possible that there are several such environments[2,3] There is definite evidence, for instance, indicating that stratiform complexes may form at spreading centres[3,4] and thus become significant parts of the oceanic lithosphere As such they may be later tectonically emplaced along collision boundaries This suggestion is given further support by the presence of cumulate textured, layered mafic–ultramafic rocks within known ophiolite sequences[5,6] (assuming ophiolites to represent ancient oceanic lithosphere) Furthermore, it has been suggested that stratiform bodies may well characterise Proterozoic ophiolites[3] It is also significant that cumulate, layered rocks within ophiolites tend to be more deformed than other types of stratiform sequences This is the case with the Giles Complex rocks[7,8]

The near parallelism of primary and tectonic layering, the close association between thrust surfaces and the layering, the presence of thrust faults at contacts with basement granulites and the gross correlation of regional trends for the mafic–ultramafic belt and the major thrust faults[7,8], all suggest a strong tectonic relationship between the emplacement of the Complex rocks and the thrust zone This is best explained in terms of a 'subducting oceanic lithosphere' model In addition the marked similarity between 'alpine-type' flow layering and deformation and the tectonic layering observed in parts of the Complex[7,8] cannot be ignored in interpreting the origin and mode of emplacement of the sequence

The presence of granulite facies metamorphism, annealed textures and other high P/T reactions are not incompatible with the model Subduction to depths of the order of 30 km would produce pressures and temperatures (10 kbar, 800° C) within the lithospheric slab[9] suitable for the development of the observed phenomena[12]

The question then arises as to the order of events as recorded in the rocks, also taking into account the phases of folding that have affected the layering The events from oldest to youngest appear to be first, the ductile shearing, metamorphism and annealing resulting in a gabbro gneiss zone, second, the folding (F_2) of this tectonic layering[8], third, the folding (F_3) of the Complex[8] on a more regional scale, and fourth, brittle thrusting

The original model again gives the best solution The oceanic lithosphere is first underthrust to depths sufficient to produce metamorphism, annealing and so on as indicated above Further underthrusting produces a maximum compression axis parallel to the layering and gives rise to F_2 folding[11] With the development of actual collision between continental blocks a period of tectogenesis and orogenesis (uplift) results in F_3 flexuring of the subducted lithosphere, slivers of which are consequently overthrust (obducted) on to the 'active' continental block[1] The latter event produces the phase of brittle faulting (major thrust faults) and mylonitisation This sequence of events also explains the lack of a metamorphic phase in the granulites correlateable to that producing the tectonic layering in the rocks of the Complex

Moore's comment regarding the difference in metamorphic grade in the basement rocks north and south of the thrust zone is a valid one and recent work also indicates differences in structural geometry[12] This, in fact, makes a collision model even more acceptable, as recourse to the tenuous concept of 'oscillation' is no longer required

In conclusion, let me point out that the fundamental interpretation of the regional geology of the area by means of a plate tectonics model is still valid and is supported by other comments made by Moore in his discussion The distribution of distinctive tectonic elements and their correlation with such a model remains the essential feature of the hypothesis The only real alteration is that of distinguishing as separate entities the Musgrave-Mann granulites (proto-Musgrave block) to the south and the gneiss–amphibolite terrain (Arunta? block) to the north of the thrust zone

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Ethylene and soil fungistasis

SMITH[1] has reported that ethylene is a causative agent of soil fungistasis, that its production in soil varies with organic matter and that in contrast with other work[2,3] it can occur in aerobic soil conditions His second claim is repetition of earlier work[3], more recent observations[4] suggest that soil anaerobiosis is necessary to mobilise substrates[5] for ethylene formation by soil microorganisms

We have attempted to reproduce Smith's experimental conditions to investigate further the effect of oxygen on ethylene formation in soil and the significance of ethylene as a fungistatic agent The soils used, which passed through a 0 1-cm sieve, were a chalk loam (10 3% organic matter) and a sand (1 4% organic matter) The soils were packed in open glass tubes and the water tension maintained at field capacity, that is, the moisture content of soil after it had drained from a waterlogged state on filter paper overnight The oxygen concentration in the loam was reduced from atmospheric to less than 0 1% and the ethylene concentration reached 28 v p m within 2 d at 25° C In the case of the sand, oxygen remained at atmospheric levels after 15 d and the ethylene concentration never exceeded 0 1 v p m When the experiments were repeated with soils which had been sieved to 0 2 cm instead of 0 1 cm, they did not become depleted in oxygen and no ethylene was detected When the top of the tube was sealed, however, the concentration of oxygen decreased and ethylene was detected

Whereas it has clearly been shown that ethylene can be fungistatic aerobically[1], it is neither fungistatic to nor metabolised by an ethylene-producing soil fungus, *Mucor hiemalis*[6], further, there is no evidence that it is fungistatic in the soil under the conditions in which it is normally formed, that is anaerobic, since it is almost certain that anaerobiosis is itself fungistatic[7]

Ethylene formed in anaerobic zones, however, could diffuse to and act in aerobic zones if it were not lost by further diffusion or metabolism[8] Germinating seeds also produce ethylene[9], presumably by an aerobic process, and this could be important in the seedling's resistance to attack by fungal pathogens

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DR SMITH REPLIES—Most of the points raised by Lynch and Harper have been clarified by our latest research[1] We have shown clearly that spore-forming anaerobic bacteria are the major producers of ethylene in soil, that ethylene is produced in anaerobic microsites in even relatively dry soil, that the anaerobic microsites result from the activity of aerobic microorganisms, and that the aerobes, in turn, are inactivated by the ethylene diffusing from the anaerobic microsites In essence, we have described a self-regulating cycle in soil that controls microbial activity

Other soil microorganisms, including *Mucor hiemalis,* may produce ethylene but the quantities will be limited by their own sensitivity to ethylene as most soils are fungistatic most of the time Also, our research has shown that ethylene production in soil is relatively insensitive to heat treatments, which rules out *M hiemalis* as a major producer because it is killed by even mild heat treatments

Claims that fungi are insensitive to ethylene must be interpreted carefully because in my original paper it was pointed out that the fungistatic action can be overridden by nutrients Thus, results obtained with ethylene in pure culture cannot be extrapolated to unsterilised soil

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tRNA and ageing

HOFFMAN and McCoy[1] have reported on the nucleoside composition of tRNA from mature and old mosquitoes and mature and old mice They reported that no alterations in the total population of tRNAs occur during ageing The authors "point out the danger of interpreting chromatographic changes in tRNA or changes in activity of tRNA methylases in terms of the final degree of modification without actually analysing the tRNA" I could not agree with them more but write to point out that they did not analyse the tRNAs whose chromatographic profiles are changed They analysed the crude mass of tRNAs which in metazoa may number more than 100 The isoaccepting tRNAs which differ in a variety of systems such as tumour tissue and differentiating tissue, are invariably very few in number and often they are minor species For example, it has been shown in Ames' laboratory that in *Salmonella* tRNA$^{\mathrm{His}}$ is the control agent for the histidine pathway operon In a mutant in which the operon is always open with the consequent constitutive status of the pathway, there is an isoaccepting tRNA$^{\mathrm{His}}$ which differs from its counterpart in the wild type by the lack of conversion of two Us to ψs[2] If we assume only 64 tRNAs in *Salmonella* and an average of four ψs per tRNA no analytical method could have established the absence of two ψs out of 256 Under the circumstances, a mass analysis of tRNA mixtures in which the preponderance of the population has not changed is meaningless

The senior author has shown earlier in aged mosquitoes a changed elution pattern in but five tRNAs[3] Only an analysis of any of these from young and aged mosquitoes would have any meaning This, of course, is technically impossible at present The authors refer to earlier work by Frazier and Yang who found no difference in elution patterns of tRNAs in young and old mouse liver[4] An examination of that paper reveals that only nine tRNA profiles were compared and most of these were done with the RPC-2 system which is known to have inadequate resolving power for the separation of isoaccepting tRNAs May I emphasise again to those who are not conversant with this field, that a very few, indeed sometimes but one regulatory tRNA, can have profound physiological effects Thus, for example, Jacobson has shown that a single tRNA can control the eye colour of *Drosophila*[5]

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DR HOFFMAN REPLIES I concur with Dr Borek when he emphasises that a deficiency in one modified nucleoside in one regulatory tRNA can have profound physiological effects I contend, however, that if that deficiency is due to altered activity of a tRNA-modifying enzyme, then all tRNAs which are substrates for that enzyme will show the same nucleoside deficiency as the regulatory tRNA It is then quite likely that nucleoside composition analysis of total tRNA would show a significant deficit in one compartment

The work of Singer *et al*[1], which Dr Borek quotes, is an excellent example It is true that lack of conversion of two Us to ψs in the tRNA$^{\mathrm{His}}$ of the *his T* mutant of *Salmonella* is directly responsible for constitutive derepression of the histidine operon It is equally true, however, that many other *his T* tRNAs lack this conversion Singer *et al*, showed directly that tRNA$^{\mathrm{Tyr}}$ and tRNA$^{\mathrm{Leu}}$ I and II from *his T* have altered chromatographic mobility when compared with the wild type They also pointed out that nearly half (5/11) of the *E coli* tRNA sequences known at that time contained ψ in the same position as in wild type *Salmonella* tRNA$^{\mathrm{His}}$, presumably as products of a *his T*-like enzyme If one applies that fraction to Dr Borek's assumed 64 *Salmonella* tRNAs, then in *his T* mutants 32 of them would each be missing two ψs for a total of 64 (not two) out of 256, a 25% deficit in ψ which would be readily apparent in a comparison of the nucleoside composition of total tRNA from *his T* and wild type *Salmonella*

Dr Borek also refers to work on the tRNA involved in suppression of the vermilion mutation in *Drosophila*[2] In a detailed study of the mechanism of this suppression, Twardzik, Grell and Jacobson[3] suggest that it is due to lack of an enzyme which modifies tRNA$^{\mathrm{Tyr}}$ They also state that "other tRNAs may be expected to be altered by the hypothetical modifying enzyme" Again I would predict that nucleoside composition analysis of total tRNA from the suppressor strain would show one component significantly deficient in comparison with wild type *Drosophila* tRNA

Our work on tRNA and ageing has been strongly influenced by Dr Borek's pioneering research on tRNA methylases, in particular the report by Mays, Finch and Borek[4] on the decline in activity of these enzymes during senescence of rodents In that report tRNA methylase activity in crude protein fractions, as measured *in vitro* by methyl saturation of total *E coli* tRNA, declined with age This decreased activity was attributed to increased glycine N-methyltransferase activity which would produce S-adenosyl-homocysteine, an inhibitor of tRNA methylases The methods used and the discussion given in this paper were directed toward total tRNA methylase activity and hypomethylation of total tRNA during ageing Based on this and my above discussion, I still maintain that it was proper for us to search for deficiencies of modified bases in total tRNA as a function of age Having found no such deficiencies, I must stand on our previous conclusion that tRNA modifying enzymes have no significant role in biological ageing[5]

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[1] Singer, C E, Smith, G R, Cortese, R, and Ames, B N, *Nature new Biol*, **238**, 72 (1972)

[2] Jacobson, K B, *Nature new Biol*, **231**, 17 (1971)

[3] Twardzik, D R, Grell, E H, and Jacobson, K B, *J molec Biol*, **57**, 231 (1971)

[4] Mays, L L, Borek, E, and Finch, C E, *Nature*, **243**, 410 (1973)

[5] Hoffman, J L, and McCoy, M T, *Nature*, **249**, 559 (1974)

reviews

SESPA, SCRAM, AIAA *et hoc genus omne*

Scientists and Public Affairs Edited by Albert H Teich Pp xi+315 (MIT Series in Comparative Politics) (MIT Cambridge and London, 1974) $14 95

THIS highly readable volume has, in essence, three themes scientists *en masse,* scientists as politicians and politicians as scientists These themes are adumbrated by five authors, eased to the reader's attention by a general preface, five individual forewords by as many pundits, and a postface It is an intriguing practice perhaps the run-of-the-mill multi-author scientific text would be more digestible if consumed with an antipasto of comprehensible individual forewords!

The first essay, by Daniel Rich, is concerned with the ethos and functioning of (American) scientific and technical societies For purposes of exemplification he depends especially on two bodies concerned with meteorology (AMS) and aerospace (AIAA), with glances at physicists, chemists and others One hotly contested theme is the role of such societies in connection with professional status Whereas engineering societies, and more especially legal and medical bodies, have a professional licensing function, scientific societies, in America at least, initially fought shy of this but are beginning to follow suit It is most interesting to see this contentious topic dealt with by a competent political theorist who casts a cold eye on professional pretensions A few quotations will convey the flavour "Competition is anathema to professionalism " "The AMS code [of professional ethics] rarely has been enforced" "The public may be protected [by a code] but the profession is also protected by the fiction that all licensed professionals are competent and ethical until found otherwise by their peers"

Another theme discussed by Rich is the relationship between the membership, the elected officers and the permanent officers of a scientific society He believes that the form of democracy often exists without the substance the permanent staff are apt to have disproportionate influence Unlike the Civil Service so bitterly attacked by Marcia Williams, however, it seems that the political 'masters' of the full-time officers are quite content with the arrangement, because generally there is little pressure for change American scientific societies are only slowly beginning to become involved with national politics (the third theme of the essays) and there is strong resistance to such involvement Possibly, when this trend develops, as it certainly will, there will be more conflict between elected and appointed officers

The second essay is by Anne Hessing Cahn, and concerns the involvement of American scientists in the controversy that raged through Washington a few years ago about the proposed deployment of anti-ballistic missiles Her study was based upon detailed interviews, or correspondence, with a sample of 152 leading scientific participants in the controversy identifying the sample seems to have been a research exercise in its own right The 152 scientists are classified in all sorts of ways—according to disciplines, type of employment, 'distinction' measured in various ways (including the number of citations of their papers), connection with the political establishment, political views, and of course their attitudes to the ABM, and all sorts of cross-correlations are established The interest of this chapter lies at least as much in the individual vignettes and insights presented in vigorous prose, as in the general conclusions drawn, and it well deserves reading The 'insiders', including several members of PSAC, the President's Scientific Advisory Committee, were deeply indignant at the way that their advice was either not requested or else ignored, and it seems that though formal advice to the president is protected by secrecy, several advisers came to think that they were entitled to publicise their personal views The most active campaigners, however, were 'outsiders'—that is, people neither working in the missiles industry nor yet in the inner advisory circles in Washington—opposed to the ABM, some of them were hostile to 'insiders' as a class (*vide* Professor Dash's bold assertion "Inside advisors are prostitutes") The outsiders' influence seems to have been, not on the executive, but on the general public, whom they energised to form active groups like SCRAM (Sentinel Cities Reject Anti-Missiles) and on members of congress One is driven to wonder whether British activists could reasonably hope for a comparable effect by convincing members of parliament

David Nichols next presents an essay on the various bodies which were set up specifically for scientists, either in isolation or in cooperation with non-scientists, to exert political pressure This essay returns to the theme touched on in the first chapter, the debate within the conventional societies as to the appropriateness of political action under their auspices (It seems, from both chapters, that physicists are still in the van of political activists) Up to now, most political action seems to be the preserve of specially constituted bodies such as the Federation of American Scientists (FAS), or Scientists for Social and Political Action (SESPA) These range from properly organised and financed lobbying bodies such as FAS to bodies whose principles will sound very familiar to academics nowadays, SESPA leaders assert that their group is a "non-organisation—a group with no officers and no constraints on membership", for "if groups are to struggle against non-participatory, undemocratic structures, it is necessary that they don't replicate such structures in their own organising" Such bodies often prefer concentrated local action Nichols, who is sceptical of the efficacy of such groups in influencing the executive branch (he does not discuss the legislature) remarks "How local action, not to mention local action by a non-organisation, can combat a ruling elite is, to say the least, not self-evident" There are lessons to be learnt by comparing Nichols's evidence and conclusions with those offered by Anne Cahn in the preceding chapter

Albert Teich next offers a fascinating survey of the attitudes and modes of life of the multi-national scientific staff of a number of European laboratories, foremost among them the ESTEC (space) laboratory in the Netherlands, CERN near Geneva, and Ispra, a former Euratom laboratory by Lago Maggiore in Italy They range from the high morale and well-defined function of CERN to the disorganisation and uncertainty of Ispra, both of which emerge clearly from many interview statements This chapter does not lend itself to summary, but makes most instructive reading, especially for those whose attitudes to European integration have congealed The one generalisation which can safely be made is that national scientists readily acquire a European outlook in these laboratories (even the indifferent ones), even most of the British, poor linguists that they still are, become more European in outlook when exposed to high concentrations of 'foreign' colleagues

The last chapter, by the late R David Gillespie, is devoted to the politics of cybernetics in the Soviet Union Norbert Wiener's pioneering ideas of the forties first ran foul of the Great Scientist's henchman, Zhdanov, on grounds based partly on the orthodoxies of dialectical materialism, and partly on the not-invented-here syndrome Later, cybernetics became part of Soviet orthodoxy In the words of David Lerner's introduction, "Gillespie charts the shift of official policy from dogged antagonism to euphoric embrace" This is a piece of very solid detailed politico-scientific history

In the postface (this must be one of the few nearly Latin words invented in the twentieth century!), Eugene Skolnikoff outlines the increasing involvement, since the Second World War, of MIT in the field of science and public policy

Overall, these essays, which although properly quantitative in approach are refreshingly clear and free of sociological jargon, go down as easily as a dish of oysters In consuming them, however, one should carefully extract the pearls of wisdom which one will certainly encounter in the process

ROBERT CAHN

Statistics in ferment

Theory of Probability A Critical Introductory Treatment Vol 1 By Bruno de Finetti Translated by Antonio Machi and Adrian Smith Pp xix+300 (Wiley Series in Probability and Mathematical Statistics) (Wiley London and New York, April 1974) £7 50

THE foundations of statistics are in dispute, and have been for many years Scientists for the most part think they need—and believe they can have—an objective language of statistics by which they can evaluate and communicate their results Usually they use significance tests, estimation procedures, and confidence intervals, and are happy to overlook the fact that most enquirers into the logical basis of this so-called 'repeated-sampling' methodology have concluded that it is unsound R A Fisher, geneticist as well as statistician, H Jeffreys, geophysicist, and I Hacking, philosopher, have been three outstanding critics Fisher, on whose early work much of the repeated-sampling development relies, was fulsome in his condemnation of later developments, and in *Statistical Methods and Scientific Inference* (1956) sought to stem the tide, allowing, apart from the simple test of significance, only those repeated-sampling methods which he thought made full use of all the information in the sample Not only was he unable to provide a unitary method for statistical inference he formed the opinion that no such method was possible Jeffreys, who had never regarded the repeated-sampling theory as more than a passing aberration, developed in *Theory of Probability* (1939), an account of the objective Bayesian theory, in which the probabilities of hypotheses are modified by the likelihood obtained from experimental results, according to Bayes' Theorem Hacking, surveying the whole field in 1965 in *Logic of Statistical Inference,* opined that there was much sense in what both Fisher and Jeffreys had to say, especially in criticism of the repeated-sampling theory, and concluded that the one element common to both their philosophies—the likelihood—could be elevated into a method in its own right, and, furthermore, that it could be extended a little into the repeated-sampling domain to give a satisfactory explication of the core of Fisher's fiducial theory and Jeffreys's theory for the estimation of location and scale parameters

As if this was not controversy enough, there are those who say that an objective theory of scientific data analysis and reporting is impossible (and who can blame them for pointing to the existing confusion as evidence?) Though the point of view has its origins in the writings of J M Keynes and F P Ramsey, its principal exponents have been L J Savage (*The Foundations of Statistics,* 1954) and Professor Bruno de Finetti In the present volume the latter offers the first half of his "necessary document for clarifying one point of view in its entirety"—the subjective Bayesian point of view The work was first published in Italian in 1970, and for English readers has been divided into two (as well as being most admirably translated)

Nobody should attempt to judge this work without prolonged reflection It must be bought, read, and then left lying around so that it can be assimilated over the years No book on statistical inference and the foundations of probability ever persuaded anybody directly, for we all make up our own minds by subjecting them to a thousand subtle influences Only once we are persuaded do we see the virtues of a clear and logical development, Professor D V Lindley may be cited as an example, for he writes, in a foreword to this volume, 'that it is a book destined ultimately to be recognised as one of the great books of the world' A reviewer, mindful of Pascal's wager, might be well advised to agree, but I have my doubts De Finetti's volume 1 presents only half his case, and since his discussion of inductive reasoning and statistical inference is not reached until towards the end of volume 2, we must bide our time What is already certain, however, is that de Finetti is giving a masterly clarification of 'one point of view in its entirety', raising the level of discussion to unsurpassed heights I await volume 2 with impatience

A W F EDWARDS

Population structure

Methods and Theories of Anthropological Genetics Edited by M H Crawford and P L Workman Pp xviii +509 (A School of American Research Book) (University of New Mexico Albuquerque, December 1973) $20

WHAT can 'anthropological genetics' be? That part of genetics pertaining to man, usually called human genetics? No, this book is really about genetical anthropology, or that part of anthropology whose study is made more fruitful by an appeal to genetical principles and genetical information, a sibling of social anthropology and physical anthropology In particular it is the study of population structure and population relatedness using models and methods developed by geneticists, and gene frequencies as a primary source of information It arose because of the conjunction of a vast increase in the amount of genetic information available and the advent of electronic computing, and has particular topicality because of the modern discussion of racial differences

About ten years ago some geneticists with a mathematical bent started applying their techniques to some of the problems of anthropology, and began developing new techniques appropriate to the computer age Although anthropologists had earlier assimilated the pioneering work on the distribution of the human blood groups, they tended to regard this incursion with suspicion, but soon came to terms with the new approach The success of the invaders owed much to the fact that not only did the geneticists bring their own tools they also brought a wide range of statistical and computational expertise that was, for the most part, new to anthropology

This volume is a revised and extended report of a conference held in early 1971, and consists of nineteen papers (the last being a useful review of the remainder) followed by nearly six hundred references These are interesting in themselves a histogram for the years of publication shows a close fit to an exponential rate of increase with a doubling time of five years (20 in 1960, 40 in 1965, 80 in 1970), and is an indication of the impossibility of being really up to date with such a book

The volume is an excellent summary of the present state of play in the field, and would provide a good entry for anyone wishing to join in There are one or two notable absentees from among the contributors, but readers will soon be led to their work through the references It is difficult to escape the impression, however, that genetical anthropology is at a somewhat adolescent stage, reflected by methodological controversies about matters of no great moment, and by the fact that in all that has been written one can discern nothing that is really surprising, no great contribution to knowledge or understanding that takes the subject beyond the merely documentary It is possible, indeed probable, that once the field has settled down something will be forthcoming, and this book may serve as a useful appetiser

A W F EDWARDS

Pharmacology of neurones

Transmitters and Identified Neurons in the Mammalian Central Nervous System By Andris K Tebécis Pp xvi+340 (Scientechnica Bristol, May 1974) £11 50

MANY books and symposia have appeared in recent years on the subject of neurotransmitters in the mammalian central nervous system, but Dr Tebécis's monograph deals with the topic in a highly original manner

One of the most powerful techniques for studying the actions of transmitter substances on neuronal receptor sites in the brain or spinal cord involves the recording of the electrical activity of single neurones while applying transmitters and other drugs in a highly restricted manner on to the surface of the cell from which such recording is being made Such micro-applications are achieved by the electrophoretic ejection of minute quantities of chemical from the fine tip of a glass microelectrode situated near the cell surface The effects of several different substances may be tested on the electrical activity of a single neurone by using multibarelled arrays of such microelectrodes Such studies allow one to define the neuropharmacological properties of individual neurones in the CNS, that is, whether they are capable of responding to various possible transmitters, and if so to define what particular type of transmitter receptor is involved This approach can be used to determine which transmitter when applied to a neurone most closely mimics the response seen when transmitter release is induced naturally by stimulating one or other of the neurone's presynaptic connections In this way one may determine the identity of the transmitters used by various neuronal pathways in CNS These experimental approaches have been widely used in recent years, and a bewildering plethora of results is available in the literature

Dr Tebécis has undertaken the Herculean task of collating much of this information into a meaningful pattern He has been helped in this by the novel approach he has taken It has become increasingly clear that the neuropharmacological properties of different types of neurone in the CNS can vary widely Results obtained by the micro-iontophoretic application of drugs and transmitters to randomly selected single neurones in any given region of the brain are thus likely to be extremely difficult to interpret, since several different neurone types are likely to be recorded from Far more consistent results are obtainable, however, if recordings are made selectively from only one type of neurone in any given brain region This may be done, for example, by using only neurones which can be identified as responding to antidromic stimulation of their axonal bundles at some remote site in the nervous system For example, spinal cord motor neurones may be identified by their antidromic response to the electrical stimulation of motor nerves

Dr Tebécis reviews what is known from neurophysiological studies of the pharmacological properties of "identified" neurones in various areas of the CNS Successive chapters deal with the spinal cord, brainstem, cerebellum, diencephalon, basal ganglia, limbic system, cerebral cortex and retina In each chapter detailed discussion is given of the results available for each of the various identifiable neurone types from which single unit recordings can be obtained In the brain stem, for example, fifteen different categories of neurone are described In each case any biochemical or histochemical data that are available concerning the distribution of transmitter-specific neuronal pathways are also reviewed The emphasis is on the actions of acetylcholine, the excitatory amino acids, GABA, glycine, catecholamines and 5–HT The author, however, also indicates the large gaps in our knowledge of the pharmacology of cerebral circuitry, pointing out the many instances in which the identity of the transmitters used at CNS relays remains unknown The book is well illustrated, and very up to date The bibliography is extensive and will be a valuable collection of source material for those interested or involved in this field

The organisation of material in the book means that it has more the nature of a catalogue of useful information than a monograph, but the catalogue will nevertheless prove very useful to many neuroscientists The author assumes that the reader has a fairly high level of knowledge of the physiological and pharmacological workings of the nervous system, so the book will appeal mainly to a small number of scientists or graduate students with this necessary expertise and interest They will, however, be grateful to Dr Tebecis for his critical and authoritative review

LESLIE IVERSEN

Science lessons

Science for the People The Origins of the School Science Curriculum in England By David Layton Pp 226 (Allen and Unwin, London, January 1974) £3 55

PROFESSOR LAYTON's well-written book ought to be made compulsory reading for all those scientists, administrators, civil servants and politicians who have anything to do with science education

By looking at the Victorian roots of the "Science for the people" idea, Layton has exposed many of the fundamental tensions in science curricula which still plague us From the beginning there were clashes in attitudes towards both method and ideology of science teaching What was science education for? Should it teach the "science of common things", or be more concerned with "applied" goals? Each viewpoint was intimately connected with political and social ideas Through studies of Richard Dawes, J S Henslow, Henry Moseley and other educationalists, and through curriculum analysis, plus some good political history, Layton has been able to synthesise a view of the past which has direct relevance today

There are, however, several things in the book which indicate a slight historical amateurishness Anachronistic social theory is one The book claims, for example, that amongst some educationalists of the 1850s, "Elementary schools were conceived primarily as instruments of social control" Possibly true, but this is much too theory-laden for the historian's palate The general aim of the book is unhistorical, too Layton has written the first seven "historical" chapters largely as a background to an almost totally theoretical chapter 8

But this is no more than a professional quibble The book itself has a value far greater than that of most historical works And Professor Layton has done science education an extremely important service

JAMES FRIDAY

Protozoa with lashes

The Ciliates By A R Jones Pp 207 (Hutchinson London, April 1974) £3 50 boards, £2 paper

MY first impressions of this little book were similar to those of a research student who, in the local bookshop, announced that it looked dull and uninteresting But first impressions can be misleading and I subsequently discovered the written content to be of a better quality than the visual appeal

The subject matter of the book is wide including chapters on the form, fine structure, locomotion, feeding, nutrition, reproduction, growth, taxonomy, and economic importance of ciliated protoza In general these chapters are readable, interesting and up to date although in places the style is rather slack A complete chapter devoted to osmoregulation and ion control is a refreshing feature not usually included in such textbooks and reflects the personal interests of Dr Jones The book is obviously written with some enthusiasm for the subject and I found several pieces of new and useful information not included in other texts I am not enthusiastic about the general format of this series of books and in this one I would have liked to have seen more original illustrations In several places I felt that a good diagram would have made the description simpler and more concise

As in all modern textbooks there is the inevitable series of errors scattered throughout the text Some although minor could be misleading, for example, referring to the Ciliophora as a subphylum and a class on the same page, the invention of a new genus *Campanula* (for *Campanella*) and some erroneous references Although I remain unclear at whom the book is really aimed I can recommend it to all those interested in ciliates since it does make a useful addition to the small but rapidly growing set of books dedicated to protozoological topics COLIN R CURDS

Dying of cold

Temperature and Life By H Precht, J Christophersen, H Hensel and W Larcher Pp xx+779 (Springer-Verlag Berlin and New York, 1974) DM 142, $58 30

Effects of Temperature on Ectothermic Organisms: Ecological Implications and Mechanisms of Compensation Edited by Wolfgang Weiser Pp xi+298 (Springer-Verlag Berlin and New York, 1974) DM 66, $23 10

TEMPERATURE is one of the most important and easily measured environmental influences affecting living organisms Many aspects of physiology are best understood in terms of reactions to stress and, of the various factors causing stress, temperature is, perhaps, the one most often encountered Study of thermoregulation has contributed much to the concept of control theory in biology by the same token, investigation of thermal adjustment in ectothermal animals is likely to have far-reaching influence on ideas about the regulation of metabolism in general For reasons such as these, various aspects of thermobiology are today being studied extensively, with the result that new books and review articles are constantly appearing The volumes under review are the latest on the subject to appear

The first is a revised and updated English version of *Temperatur und Leben* by Precht, Christophersen and Hensel, first published in 1955 Although constructed along the same lines as its predecessor, it differs in that the number of contributors has been increased to include two microbiologists, one biochemist, seven botanists, three zoophysiologists and three human physiologists An attempt is made to treat as many problems as possible, but the main theme remains the adaptation of organisms to changing temperatures A supplementary volume on physical and chemical aspects by L Lumper, is to be published later A special effort has avowedly been made to cover, often in the form of footnotes, the copious literature that has appeared since 1955 To judge by those particular fields with which I am familiar, however, it would seem unlikely that more than about one tenth, at most, of significant contributions has been included Even so, a rather turgid compendium has been produced, probably of use to research workers for reference purposes rather than for stimulation, whose detail at the same time far exceeds the requirements of most graduate students

The various chapters have been grouped under the headings of poikilothermic organisms (microorganisms, plants, animals) and homeothermic organisms In my view there is no clear distinction between homeothermy and poikilothermy, so the classification adopted tends to obscure the relationship between homeothermy and size in animals With an unfavourable surface to volume ratio, the maintenance of a constant body temperature would be economically unacceptable for small organisms

Temperature and Life shows little evidence of the meticulous planning that clearly went into its nearest competitors such as *Temperature Regulation in Mammals and other Vertebrates* by J Bligh, (Elsevier, North-Holland Amsterdam, 1973), *Cryobiology* edited by H T Merryman (Academic Press London and New York, 1966), *Thermobiology* edited by A H Rose (Academic Press London and New York, 1967), and *Comparative Physiology of Thermoregulation*, vols 1–3, edited by G C Whittow (Academic Press New York and London, 1970, 71, 73) Of course, none of these covers quite such a wide field as the new book, although their design is far better Indeed, herein lies the nub of my criticism The study of temperature and life has long since passed the point at which it can be reviewed comprehensively in a single volume—even one of 779 pages with fine type

Wolfgang Wieser's book likewise suffers from the fact that it possesses little intrinstic homogeneity This, however, is probably unavoidable, since it represents the proceedings of a symposium held at Obergurgl, Austria, from September 4–8, 1972, at which only a restricted number of topics was covered The 25 contributions are grouped under three headings mechanisms, ecology and cold resistance but, within these broad classifications there is often little in common between one paper and the next There is also very considerable variation in quality An opening review by the editor stresses the complex "multistable system" from which the answer best suited to the prevailing or anticipated thermal régime is called into action by changes in ambient temperature This farsighted approach may well provide the kind of stimulus lacking from *Temperature and Life*

Despite the vast amount of data reviewed in both books, it is evident that knowledge of thermobiology is still superficial To cite a few examples, the mechanisms by which death results from heat or cold are very imperfectly understood They are certainly diverse, and are complicated by the fact that unrelated mechanisms within an organism may be influenced concurrently in different ways Extremes of temperature may denature enzymes, or liquify membrane lipids The injurious action of heat may also damage mitochondria, affect the coding of proteins, impair the coordination of physiological processes, disturb the functioning of endocrine glands and neurosecretion, and engender the accumulation of harmful metabolic products or an inadequacy in the transport and supply of oxygen The harmful effects of cold are equally multiple Again, little is known of the neurophysiology or mode of function of thermal receptors in animals, nor why long-term adaptation has so little effect on the properties of these sense organs It would be misleading to lump together a number of unrelated phenomena merely because they are engendered by the same thermal influence one must therefore accept the fact that no unitary explanation exists

The indexes of both volumes are restricted to subject only the titles are omitted from references cited in the first Since these number well over 4,000 this defect is, no doubt, unavoidable

J L CLOUDSLEY-THOMPSON

Systematic archaeology

Kalambo Falls Prehistoric Site Vol 2 The Later Prehistoric Cultures By Desmond J Clark With contributions by B M Fagan, M R Kleindienst and F van Noten Pp xiv+420 (Cambridge University London, February 1974) £17, $49 50

THIS is the second volume of Professor Clark's report on the archaeological sites at Kalambo Falls on the borders of Zambia and Tanzania The first covered geology, palaeoecology and stratigraphy (reviewed *Nature,* **223,** 326, 1969), the second covers the upper part of the archaeological sequence, from the Iron Age back to the Second Intermediate, about 7600 BC The third volume, still in preparation, will cover the earlier periods, ranging in date from about 25000 BC to more than 60000 BP Both published volumes reflect the modern standards required for excavation reports Very careful excavation of a number of sites has produced a remarkable range of information particularly in the environmental field, further, a number of carbon-14 dates have been obtained covering a long period, dates which are much needed in African prehistory

The upper part of the archaeological sequence, the Iron Age, covers a time span from about AD 345 to AD 1580, the last date apparently being that of the foundation of the modern settlement in the district This continuity of occupation is discussed in chapter 1, where the history of the recent Bantu settlers is dealt with in some detail Chapters 2 and 3 cover the Iron Age settlements and chapters 4 to 7 inclusive the two Stone Age complexes which precede them

The latest of these Stone Age industries has carbon-14 dates of around 1950 BC and belongs to the "Late Stone Age" of southern Africa Like many complexes grouped under this heading the Kaposwa, as Clark calls this material, is characterised by an abundance of microlithic tools, mostly crescents and triangles This Kaposwa has been compared with a number of sites in Zambia, Malawi and further south which are within the same time range and which have a comparable industry, some of these seem to be as early as 10000 BC, similar in age to the early postglacial industries in Europe The trend towards microlithic tools continued in parts of South Africa into early colonial times among groups which had retained a strictly hunting and food-gathering economy

Clark named the other stone age industry Polungu It belongs to a phase which in southern Africa is referred to as the Second Intermediate, a stage between the Middle Stone Age with industries based on a flake technology and the frequent use of prepared cores, and the Late Stone Age with microliths and a blade technology like the Kaposwa The Polungu from Kalambo Falls comes from two stratigraphic units So far only the earlier has produced a carbon-14 date, about 7600 BC —towards the end of the time range for these industries which probably go back to about 1600 BC This period was within the Late Glacial in Europe

Both Kaposwa and Polungu differ in some respects from other Second Intermediate industries, not surprisingly considering the time and distance separating them The Second Intermediate industries do, however, make a fairly homogeneous group

Clark treats all three complexes in great detail with a minute statistical breakdown and a discussion of each in relation to its place in the African sequence, thus providing an informative summary of later African prehistory He has also given a great deal of space to his own views on classification so his terminology is clear Every ounce of information possible has been extracted from the excavation and presented in a very systematic manner with an excellent standard of book production I have only very trivial complaints plate 98 seems of little value and the caption of plate 97 could have been a little clearer

J WAECHTER

Biology called to account

The Scientific Conscience Reflections on the Modern Biologist and Humanism By Catherine Roberts Pp xii+130 (Centaur Fontwell, Sussex, July 1974) £2 50

MANY people have uncomfortable feelings of moral ambivalence when they consider the aims, practices and ideas of much current science, and particularly of the self-styled "new biology" The leading figures in this domain may be admired or envied for their energy, cleverness, and social success, but few would look to them as exemplars of the primary human virtues The modesty of a Darwin, the personal courage of a J B S Haldane, the altruism of an A R Wallace, the sense of public responsibility of a Pasteur, or the dedicated persistence in the face of poverty or ill health or both, displayed by many humble naturalists of the last century, are all qualities hard to match among the biological celebrities of our day It is scarcely accidental that several recently published books on the philosophy of biology make no reference to ethics at all, but treat science as an entirely autonomous domain The moving spirit of modern biology has a decided resemblance to that Mephistopheles who in the prologue to Goethe's "Faust" presented himself before God in true Popperian fashion as "the spirit that denies", and later in the play was constantly urging Faust to "the effecting of all things possible" by demonic aid

Dr Catherine Roberts, a microbiologist turned to classical Greek studies and philosophy, has gathered together a number of essays, the chief unifying factor among which is that they are all directed against various aspects of the Mephistophelean spirit in modern biology The latest of the essays dates from 1966, so that we do not have Dr Roberts's reactions to *The Double Helix* of J D Watson, which might have been interesting Her own standpoint, nowhere clearly stated in this book, would seem to be a form of neo-Platonism like that of Kathleen Raine, and very definitely committed to the cause of God as against Mephistopheles Her basic attack is directed against the whole concept of science as a completely autonomous activity Despite the title, her argument implies that there cannot really be such a thing as a 'scientific' conscience, if we make moral judgments and valuations, we cannot do this quâ scientists, but only in what she considers to be our higher capacity as fully human beings

The essays are essentially separate, and inevitably somewhat overlapping, they express, at times with some eloquence, a consistent point of view, and one which is likely to gain more adherents in future, but do not attempt a systematic intellectual exposition of it—for this, with engaging modesty, Dr Roberts refers her readers to Michael Polanyi's well known *Personal Knowledge* (1958) The whole book has what used to be thought of as a 'feminine' quality, basing itself more on an appeal to the heart than on coercive rationalisation Her opponents will doubtless deny the validity of any such appeal, and take credit for their own heartlessness We may well wonder for how long it will be possible for such people to pursue ambitious scientific careers, financed at the expense of taxpayers, half of them women, and most of them with old fashioned human hearts

R A CROWSON

Microbes and men

Microbes and Men Producer Peter Goodchild BBC2, Wednesday 9 55 pm

FOR their newest medical/scientific effort, BBC television have homed in on four of the founding fathers of modern medicine and bacteriology, and by means of carefully reconstructed dramatised documentary have tried to tell it as it was Unfortunately, in the process they have failed to enliven these undoubtedly fascinating characters, Semmelweis, Pasteur, Koch and Ehrlich

Locations, medical instruments, crucial experiments and the appalling conditions in hospitals at that time are painstakingly reconstructed, and the commentary linking the episodes of dramatic action is lucid, informative and accurate They have foregone the image of the great scientist and have faithfully documented the petty squabbles, the failures and the obtusenesses as well as the brilliant insights and triumphs

The fact that in spite of all this the programmes never come alive may be a fault inherent in the presentation itself Patches of dramatic dialogue are interspersed with the commentary, which although necessary to convey all the hard facts and point the moral in the 55 minutes allowed for each episode, throws an air of unreality over the whole proceedings, especially as the marriage of commentary to picture is sometimes heavy-handed

The first programme of the series, on the Hungarian doctor Ignatz Semmelweis, screened this week, may, in fact, be the best Semmelweis was an exceedingly complex character, especially in his failure to follow up his brilliant work on the origins and transmission of puerperal fever, a disease which killed one in three women in childbirth in the 19th century lying-in hospitals As one character says to Semmelweis' wife in the 'authentic' context of the cosy drawing room, "Science is moving, and I want to make sure he doesn't get left behind" The programmes are in fact littered with this sort of aphorism, usually delivered in ringing tones The overall effect, for all its undoubted authenticity, is remarkably reminiscent of the great 'lives of the composers' *genre* of films

The BBC have seen fit to screen the series relatively late in the evening although the programmes would be eminently suitable for children and would probably be much enjoyed by them Perhaps the BBC thought that the blood and gore, especially in the first episode might be off-putting, but children are usually far more resistant than the adult audience for whom it is obviously intended

A book of the series (*Microbes and Men*, £2 50) will be available next month

ELEANOR LAWRENCE

obituary

H. Taylor

SIR HUGH TAYLOR, former graduate school dean and chairman of the chemistry department at Princeton University for 25 years, died at Princeton on April 17, 1974 at the age of 84

Born in St Helens, Lancashire, he received his University training at Liverpool under the tutelage of Sir Frederick Donnan, graduating in 1909 and earning a masters degree in 1910 In 1912 and 1913 he studied reaction kinetics, the first year at the Nobel Institute at Stockholm with Svante Arrhenius and the second with Max Bodenstein at Hanover Technische Hochschule In 1914 he was awarded a doctorate at the University of Liverpool He went from there to Princeton University, where he spent the rest of his life except for two years during the First World War when he worked in the United Kingdom

In Princeton, Taylor progressed rapidly through the early professional grades becoming a professor in 1922 and Chairman of the Department of Chemistry by the time he was 36 He served in this capacity from 1926 to 1951 He also served with distinction as Dean of the Graduate School from 1945 to 1958 A person as effective as Sir Hugh has no opportunity to retire After 1958 he continued his dedicated interest in graduate education serving as the first president of the Woodrow Wilson National Fellowship Foundation, the Princeton-based foundation supporting quality in higher education

Sir Hugh's influence on kinetics has been felt worldwide, especially as applied to catalysis which is a theme running through his 300 scientific papers and his books The book he wrote with E K Rideal *Catalysis in Theory and Practice* published in 1919 grew out of work by the authors in Britain during the First World War and was widely used His contributions to photochemistry, radiochemistry and chemical kinetics were likewise fundamental During the Second World War, his pioneering efforts in preparing heavy water and diffusion barriers were of great importance to the atomic bomb project

No picture of Sir Hugh would be complete which left out his generosity, his intense vigour and his decisiveness He usually emerged from his office just slightly slower than running In the seminars which he presided over so superbly, no one could present more quickly and clearly the gist of the problem at hand and place it in proper perspective With this went a warm enthusiasm and an appreciation of good work by others that made reaction kinetics, and chemistry in general, blossom profitably at Princeton

Announcements

Erratum

In the article "Activation of suppressor T cells by tumour cells and specific antibody" by R K Gershon *et al* (*Nature*, **250**, 594, 1974) the end of one sentence and the beginning of the next were inadvertently omitted from the penultimate paragraph The third and fourth sentences of that paragraph should read 'It is well known that the immunogenicity of antigen is highly dependent on the physical state in which it is administered[19] Antibody can affect the physical stage of antigen[20] and in so doing, perhaps, change the T cell response to it'

Reports and Publications

Great Britain

The Zoological Record 1970, Vol 107 Section 6 Vermes Part C Conodonta, Fossil Vermes Trace Fossils Compiled by S Ware Pp 22 £3 65 1970 Vol 107, Section 13 Insecta Compiled by the Staff of the Zoological Society of London Pp x + 729 £12 15 (London The Zoological Society of London, 1974) [107

Philosophical Transactions of the Royal Society of London B Biological Sciences Vol 267, No 890 Biomechanics of *Pteranodon* By Cherrie D Bramwell and G R Whitfield Pterodactyls Past and Present By D M S Watson D M S Watson s Notes on Pterosaurs By Cherrie D Bramwell and G R Whitfield Pp 503–592 + plates 24 and 25 (London The Royal Society, 1974) [117

Other Countries

Lawrence Livermore Laboratory in Brief Pp 24 (Livermore California Lawrence Livermore Laboratory, 1974) [87

Smithsonian Contributions to Zoology No 139 Gammaridean Amphipoda of Australia, Part II By J Laurens Banrard Pp v + 148 $2 20 No 159 Clinid Fishes of Chile and Peru with Description of a New Species, *Myxodes ornatus* from Chile By John S Stephens, jun and Victor G Springer Pp 24 70 cents (Washington, DC Smithsonian Institution Press, 1973 and 1974 For sale by US Government Printing Office) [87

nature

Volume 251 September 27, 1974

Going on about race

CONSTANT LAMBERT was an opinionated but highly readable commentator on the musical scene of the 1920s and 30s, of which he himself was a part. In *Music Ho! A Study of Music in Decline*, he wrote thus of Russian symphonic music based on folk origins—'. . . the whole trouble with a folk song is that once you have played it through there is nothing much you can do except play it over again and play it rather louder!' The conflict between those who see a genetic component in white/black IQ differences, and those who do not runs some risk of becoming in science what the folk song became in symphonic music. The latest action replay of the controversy was performed in London last week at the Institute of Biology's seminar on Racial Variation in Man.

The conflict is actually a three-cornered affair between those who say 'nature' dominates, those who say 'nurture' dominates, and those who cry racism. These last who vilify the 'nature' proponents as racists—and they managed a modest demonstration outside the hall last week—should be aware of some dangerous pitfalls.

- It is no good simply decrying the IQ test as such. There is no doubt a substantial degree of disaffection with IQ as a valuable measure of anything useful. Fun can be poked at the skills which it seems to encompass; much can be made of the success of those with modest IQs, and the failures of those with astronomical ones; it can be accused of dividing children at too early an age into sheep and goats, but the fact remains that it is a widely used measuring rod. Further, as Professor Thoday put it in *Nature* last year, 'concerning within-group differences . . . relative performance in IQ tests correlates with relative performance in aspects of education that are highly relevant to success in, and the needs of, modern societies.'

- It is no good denying the existence at present of between-group differences in mean IQ. They are real and it serves no useful purpose to pretend that they are the result of errors of measurement or that they are trivial.

- Lionisation of those who have found a low degree of heritability of IQ is wishful thinking and an utterly unscientific approach. It would do the cause of social responsibility no good to be wedded to an attractive but maybe wrong conclusion.

In short, catch phrases such as 'Smash the racist IQ test' and 'Nurture not nature' are no way to approach the problem. But problem there is. It is difficult to escape the conclusion that those who go on saying that heritability is high are feeding racist tendencies, however inadvertently and seemingly innocently, and are supplying an apparent scientific basis for those who wish to justify racial discrimination. Many scientists would, of course, contend that the evidence on which the present debate is being conducted is not such as could correctly be termed scientific, but the general public, unlikely to appreciate the finer points of the internal squabbles of science, will in the end absorb the message that is repeated most frequently and with the most crescendo.

Educational policy-makers may then be under considerable pressure to introduce some sort of *apartheid* system which tries to partition off by racial group in the interests of uniformity. However neat and justifiable such a system might seem on the face of it, it would be going in the wrong direction in two distinct ways: the educational experience should be more than just intellectual pump-priming, it should now more than ever help to reduce societal divisions; and to educate on the basis of group membership rather than individual capability is to waste the diversity of talent which sometimes seems the main asset of society.

The scientist faced with a discovery which is controversial and maybe unpalatable to some has an awesome responsibility to handle it correctly, and he should be prepared if necessary to devote the rest of his scientific life to ensuring that the proper use is made of the discovery. Would racial segregation in education be a proper use for apparent high heritability of IQ?

It is simply not possible, having unearthed a potentially explosive piece of information to sit on the fence in the name of scientific objectivity. Those who do not take every opportunity to minimise the risk of the subsequent explosion are actually helping it to come about. And those who keep raising the inflammatory issue must be presumed to want to speed the explosion.

A hundred years ago

Mist Bows

ON Sept. 14 I was driving from the Lizard just after sunrise with Mr. Lugg of Manaccan. A thick mist covered the fields and moorland. The tops of the farm buildings and corn stacks and the church towers were visible above the sea of mist which, matted on the ground, gave the entire country the appearance of being covered with snow. About 6.30 A.M. the sun was bright on our right hand, and on the left we saw a halo of prismatic colours forming a distinct circle of rainbow at a little distance from and encircling the shadows of our heads, and only broken where the shadows of our bodies interposed. This appearance lasted for ten minutes, and our shadows with their attendant bow showed brightly against the mist background as we passed hedges and fields, and kept pace with us like "the mist raised from the plashy earth" by the hare in Wordsworth's poem,

"That, glittering in the sun,
Runs with her all the way wherever she doth run."

We afterwards opened a valley terminating in an extensive moor, when the mist appeared as a sea of prismatic colour extending to the horizon. About 7 A.M. we saw a perfect bow free from any prismatic colour, both ends of which terminated in the field immediately to our left.

My companion, who is constantly driving about this district in early morning, says he never before saw similar phenomena.

Lizard Signal Station, Sept. 16 HOWARD FOX

From *Nature*, **10**, 438, October 1, 1874.

Towards a European Space Agency

SINCE April 1 this year we have awaited the establishment of the European Space Agency (ESA). The new single organisation will take over the functions of the three existing bodies—ESRO, ELDO and the ESC*—to become the NASA of Western Europe. ESA will be responsible for running not only the scientific and application satellite programmes of ESRO but also the three new ventures decided a year ago by ten European space nations—the Spacelab orbital laboratory, the Ariane launcher and the Marots maritime satellite. Each of these three new programmes will be developed under the respective sponsorship of Germany, France and the UK. The rules of participation in the programmes will also be changed by the new convention of ESA. As before, a participating country will have work allocated to it by the Agency in proportion to the amount of cash it subscribes. But now member-states will also have the right to contribute such sums as they feel necessary, and to opt out of specific Agency programmes which they don't find attractive.

The creation of ESA has been progressively delayed throughout this year. The ten European ministers will meet again this week in Paris, but a French government source said last week that there would be no decision until mid-October. Indeed, it is absolutely impossible to foresee what could happen next month. There may be another postponement, but it is hoped that ESA should be settled before the end of the year.

The delay is caused by a fight for the leadership of ESA between the two most important contributing countries. France and Germany have both proposed a candidate as first general manager of the organisation. France is also trying to obtain the participation of the other member states to the cost of the French Guiana Space Centre, which became the launching range for the Ariane rockets. Unexpected events like the sudden death of President Georges Pompidou and the following election campaign have also contributed to the delay.

The new French government hasn't decided much about space affairs—apart from signing the Memorandum of Understanding for the joint development of the Aerosat satellites by Europe and the United States.

On current thinking, the French government should define its space policy in early October at a special council of the ministers concerned. Some official sources say it will closely analyse the space report, and the Ariane project will certainly be questioned. The former government always thought that the heavy launcher was to be "the keystone of the European space policy", considering the political and economical value of the application satellites. More than 3,500 million francs have been spent to duplicate the US heavy rocket that failed when the Europa 2 rocket blew up in the Atlantic. Now France and Germany require an American launcher to put the Symphonie communications satellite into orbit in December of this year. But the US State Department imposed such limitations to the use of this experimental satellite that its interest will be significantly reduced.

Now comes the second and decisive stage for the European rocket. In May, 1973, France proposed to jump a step with Ariane, a more powerful launcher able to place a 750 kg payload into geostationary orbit, which could be developed before the end of the decade. France picked up at least 65% of Ariane costs, which are currently budgeted at about 2,500 million francs for the development only. Ten European countries, including the U.K., are participating in this programme. Tooling up, long-term supplies and engine ground testing are now under way, and the industrial team has been selected recently.

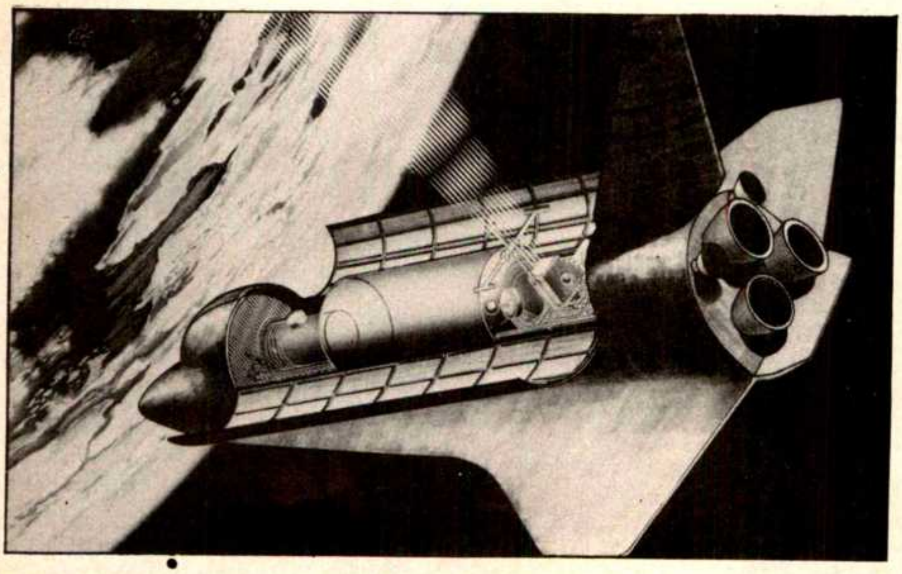
Meanwhile, last June, the French government withheld authorisation for the Centre National d'Etudes Spatiales (the technical and financial manager of Ariane) to conclude the long-term contracts of development with the main contractors. The government needs to think carefully before acting because the programme necessitates more and more spending during the next three years at the same time as the other European space activities also begin to be more costly. In spite of all these new expenses the government don't want to increase the space budget; recent reports from the French Ministry of Industry are very clear about this. In such circumstances the CNES may be obliged to reduce, postpone or even to cancel some of its activities although usually France considers that European expenses take priority.

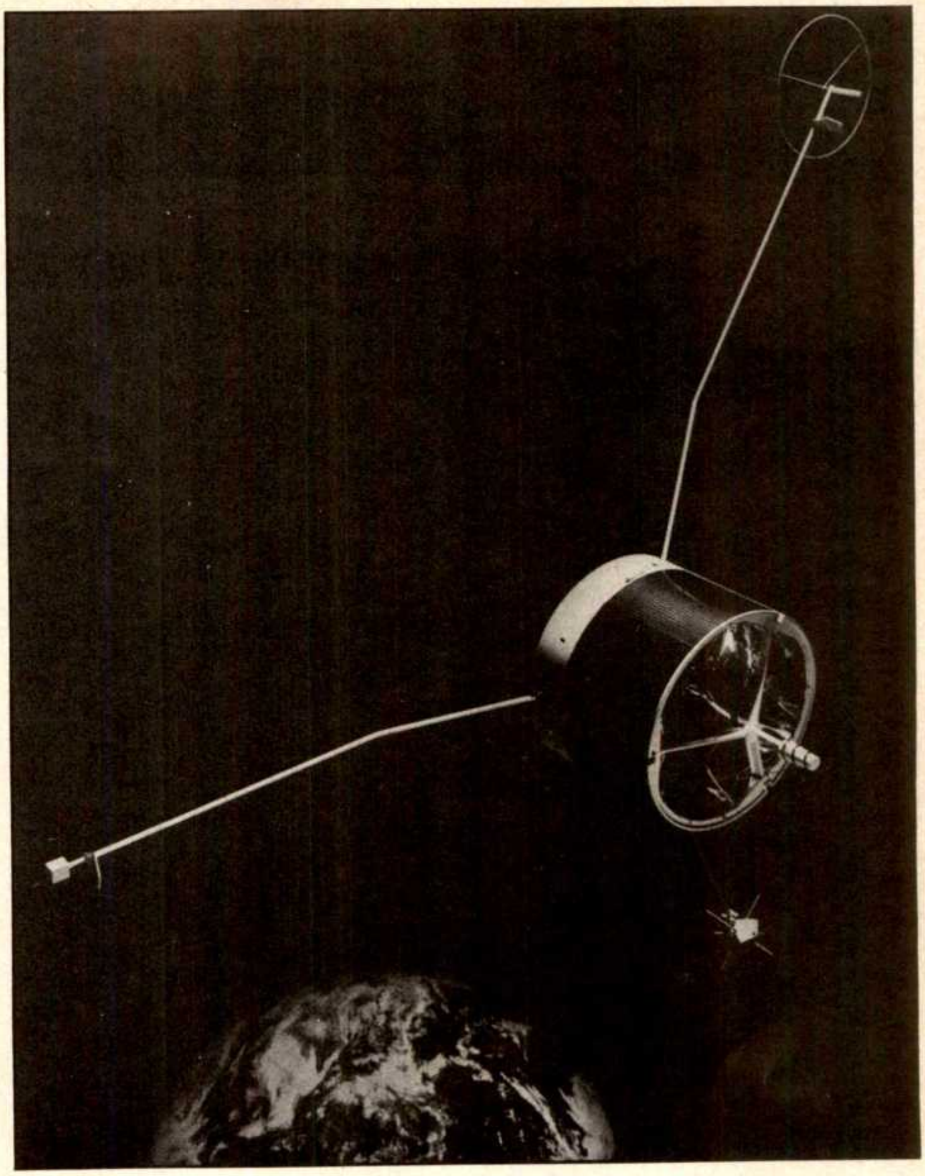
Nevertheless, the Ariane programme could be in danger. The 200-ton three-stage launcher will be the largest rocket built in Europe. But it will be relatively expensive and its market seems less important than expected. A joint study by French and Germans anticipated 35 to 50 launches in ten years (1980–90). But it was never published, and the specifications for the launch range consider only two firings per year during the same period, i.e. less than half the forecast.

The real reason for the difficulties is a political, economical and cultural one: the French desire to build "the launcher of European independence". But it is not certain that the new government will follow its predecessor on this policy. President Valery Giscard d'Estaing has based his policy on change, and on the evidence of the past three months he has a habit of making surprising decisions. For the present government to drop the idea of backing the launcher would be the worst thing possible for the French aerospace industry.

Despite these troubles, the other programmes are running well. Spacelab, which will also be a long-term develop-

Spacelab: first flight planned for 1980.





International Magnetospheric Explorer (IME): to be placed in orbit in 1977.

ment with significant spendings, is going on. This 400 million dollar programme will be a truly international space venture under the leadership of Europe. Developed by 10 countries—all 10 members of ESRO except Sweden but with Austria—the Spacelab will be a part of NASA Space Shuttle Transportation System. European industrial and technical teams are in permanent contact with NASA officials over the development of this spacecraft, which will be completely dependent on the Space Shuttle Orbiter.

It is a versatile manned space laboratory in which scientists and engineers with only a short training as astronauts will be able to live and work for periods of one week to one month in weightlessness. The crew will perform different tasks: scientific missions, Earth resources and weather surveys, communications and other technology experiments. The orbital laboratory has two main parts: a pressurised module connected by an airlock to the Orbiter crew cabin and a pallet which supports instruments exposed to the vacuum of space.

NASA has ordered two Spacelabs from ESRO. The first flight of Spacelab is planned for April 1980, with the seventh flight of the Shuttle, on a mission not yet defined. More than 200 experiment proposals have already been received by ESRO from the European scientific community for this flight.

The next two European scientific satellites will be in space within a year. The COS-B satellite will be launched in April or May 1975 to study gamma rays, and the GEOS satellite will be put into the geostationary orbit in September, 1976, to investigate plasma distribution, energetic particles and electromagnetic fields.

In December, 1976, NASA will launch the International Ultraviolet Explorer satellite (IUE), a joint US-UK project in which ESRO participates. The next project will also be a cooperative one between NASA and ESRO. The ISEE satellite (formerly IME-D) is due for launch in September, 1977, with a brother American satellite from the same launcher to study the magnetosphere. For mid-1979, ESRO is preparaing the highly eccentric lunar occultation satellite (EXOSAT) that will map the X-ray sources.

Since 1970 13 scientific mission proposals have been submitted to ESRO, but only one or two will be retained in early 1976 for development. All these missions could be realised with the Spacelab or free flyer satellites. Two-thirds of them are cooperative projects with NASA. Nine are devoted to infrared, ultraviolet and X radiation studies of the Sun and the sky. Three others are more impressive projects including the first probe to make observations from out of the plane of the ecliptic.

There is also a Pioneer–Jupiter orbiter probe and the interception of Comet Encke by the end of 1980 to proceed with the first close study of thesem ysterious space objects. Another proposal is an astrometry mission to chart more than 30,000 stars down to magnitude 17 with a precision 10 times better than the existing references. The feasibility of this project will be discussed at a special colloquium to be held next month at Frascati (Italy).

Among the application programmes, the meteorological satellite (Meteosat) will be put into geosynchronous orbit in December, 1976, to participate in the Global Atmospheric Research Program of the World Weather Watch. Three other experimental satellites are due for launch close to the end of 1977 to prepare the operational systems of the 1980s. The OTS satellite will pave the way for the European Communications Satellite (ECS) designed to provide space links for intra-European telephony, telegraphy, telex and TV relay for the European Broadcasting Union. The aeronautical satellite (Aerosat) will be the first step towards a world-wide air traffic control and communications satellite system. And the maritime satellite (Marots) will carry out experiments involving communications between ground and merchant ships to prepare the future IMCO operational system to cover the seas and the oceans of Earth. All these new European satellites—like the seven built over 10 years by ESRO—will be launched by American rockets from the United States.

*ESRO is the European Space Research Organisation; ELDO is the European Launcher Development Organisation formerly responsible for the launcher Europa 2, and ESC is the European Space Conference, i.e. the forum for decisions by the European science ministers.

—From the staff of La Recherche

international news

Fruits of a Faustian bargain

by Colin Norman, Washington

Charles Colson, former President Nixon's one-time hatchet man, announced soon after the 1972 election that the Administration would take no vindictive action against those who had the temerity to oppose it. In fact, said Colson, a large federal programme was already in the works for the only state which had cast its votes for McGovern—a nuclear waste repository would be built in Harvard Square.

Fortunately, even though members of the infamous White House enemies list were heavily concentrated in the Boston area, Massachusetts won't be so lucky. That particular federal programme will go to Nevada, Idaho or Washington state.

Last week, the Atomic Energy Commission announced that sites in those three states have been shortlisted as potential locations for a temporary storage facility where highly radioactive waste material from nuclear power stations will be kept under surveillance until a safe, proven method is found to get rid of the stuff. The agency also acknowledged that it has yet to find such a method.

The announcements and acknowledgements were contained in an unusual document—an environmental impact statement in which the AEC, instead of analysing the consequences of a single, chosen programme, has floated some ideas, asked for comments on them, and said that it will take the comments into account when it makes its final choice.

Although that piece of before-the-act public consultation has been welcomed by the AEC's friends and foes alike, it will, nevertheless, do little to dampen the fierce debate over nuclear waste disposal which has been going on in the United States for several years. But it should at least help to sharpen the issues. For those who have tuned in late, a little background is in order.

The waste material in questions consists of radioactive isotopes formed in a reactor core from uranium fission. When irradiated fuel rods are removed from the reactor they contain not only unwanted isotopes such as strontium-90 and cesium-137, but also large quantities of plutonium and unused uranium which are so valuable that they are removed in a reprocessing plant and later reused as reactor fuel.

What is left is an acidic solution of waste material which contains virtually all the radioactivity from the spent reactor fuel and which must, somehow, be disposed of. Since such solutions are not the easiest things to store—in fact, there have been some serious leakages from storage tanks at AEC facilities in Washington and Georgia—the AEC began a programme in the 1950s aimed at finding a method of reducing the waste solutions to stable solids.

A method has been developed which essentially consists of depositing oxides of the unwanted isotopes on granules of aluminium oxide, and in 1970 the AEC issued a regulation which requires that all highly radioactive wastes from commercial power stations be solidified and sealed into steel canisters before being shipped to a federal nuclear waste repository.

The problem, though, is that no such repository yet exists because the AEC has had considerable trouble finding a suitable dumping ground. The trouble stems from the fact that because the reactor wastes contain extremely long-lived radioactive isotopes which will remain highly dangerous for several centuries, they must be kept isolated from the environment for thousands of years.

The most promising disposal method, according to virtually every committee and commission which has studied the matter—including the National Academy of Sciences—is to bury the waste canisters in bedded salt, which is geologically stable, provides an effective radiation shield, and is unlikely to be in contact with subsurface water.

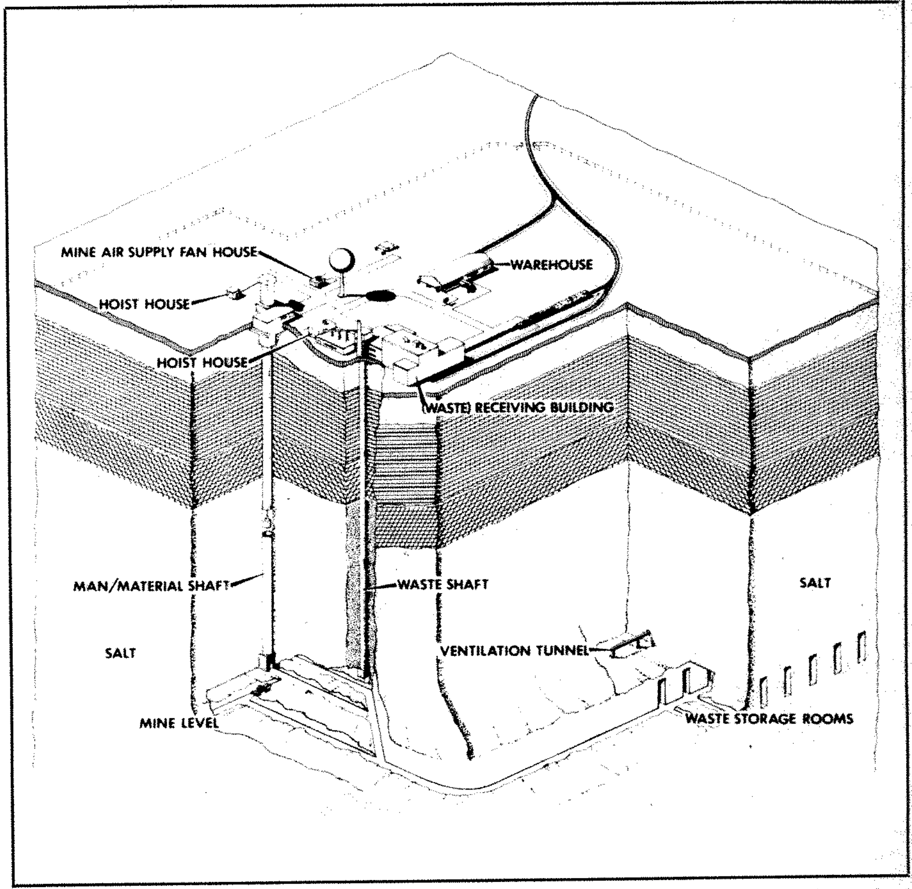
During the 1960s, the AEC found what looked like an ideal site—a disused salt mine near Lyons, Kansas—and in 1971 the agency asked Congress for funds to conduct pilot-scale operations there with a view to turning the mine into a federal repository if all went well. Fortunately, though, the project met with political opposition, Congress denied the funds and it later turned out that the area was, as one Kansas state engineer put it, "like a piece of Swiss cheese". Back to square one. Then, in 1972, the AEC announced that it was going to buy some time by constructing a storage facility above the ground in which wastes could be kept under constant surveillance while a search is made for a permanent disposal site. And that, essentially, is where last week's environmental impact statement comes in, for the AEC has now not only shortlisted potential sites, but it is also close to choosing a design for the storage facility.

In the meantime, however, critics of nuclear power have seized upon the AEC's difficulties and turned them into a potent argument against the burgeoning growth of nuclear generating capacity. They have argued that it is immoral and unwise to push ahead with a technology which will leave a dangerous legacy for many generations. The nuclear power programme should therefore be halted at least until a safe waste disposal method is found and tested. Needless to say, the AEC does not agree.

According to the environmental impact statement, the above ground storage facility will be capable, if necessary, of maintaining waste canisters safely for "at least 100 years", although, with luck, that will not be necessary for the AEC hopes to have found, tested and prepared a permanent disposal site within two or three decades.

The AEC's strategy, as outlined in the environmental impact statement, is this: It will probably choose a design and a location for the storage facility in the next few months, perhaps even in time to ask Congress for construction funds in the 1976 fiscal year budget. The facility should be ready to receive its first shipments of waste canisters by 1981, and it will gradually be extended to cope with increasing volumes of wastes towards the end of the century. By the year 2000, in fact, nuclear power stations in the United States will have produced almost a quarter of a million tons of solidified wastes.

In the meantime, the agency will continue searching for a geologically stable burial site where the wastes can be disposed of permanently. Other salt mines in Kansas, and salt and potash deposits in New Mexico are under consideration, and according to Dr Frank K. Pittman, Director of the AEC's Division of Waste Management and Transportation, some pilot-scale tests could begin at one or more potential



Bedded salt pilot plant: most promising disposal method

dumping sites in the 1980s. Pittman added, however, that although he is very hopeful that a suitable site will be found, the AEC has no "implicit faith" that such a site exists.

As for the temporary storage facilities, the environmental impact statement describes three potential designs, each of which has its own advantages and disadvantages. In terms of cost, however, they would work out at about the same—$100 million.

The essential feature of any such repository is that the waste canisters must not only be shielded for radioactivity, but they must also be kept cool because the radioactive decay gives off vast amounts of heat—the temperature at the centre of the waste canister could reach almost 1,000° C. One of the designs would use water as the coolant, while the other two would rely on air cooling.

- The water-cooled design would consist of a series of stainless steel-lined concrete vaults, each capable of holding about 500 canisters and each filled with demineralised water. The water would be kept cool by continuous circulation through a closed loop in which the heat is given up to a secondary cooling loop and dissipated to the atmosphere through a cooling tower. As well as providing cooling, the water also acts as an effective radiation shield, and since it will be constantly monitored for radioactivity, canister leakage can quickly be spotted.

The disadvantage with this design, however, is that it would require large quantities of cooling water, and corrosion may also be a problem over long periods of time.

- One of the two air-cooled storage designs under consideration—the so-called air-cooled vault concept—would consist of a series of large vaults similar to those in the water basin design, each of which would be capable of storing 500 canisters. Each canister would, however, first be sealed in a steel overpack to provide extra protection against the canister rupturing and spewing out its radioactive contents. Cooling would be provided by passive circulation of air through the vault, which would maintain the temperature at the surface of each canister overpack to about 200° C, and the thick vault walls would provide radiation shielding.

The advantage of this design is that there is virtually no possibility of breakdown in the cooling system, but it is the most expensive design and it would disturb large amounts of land.

- The third choice, the sealed cask storage concept, differs from the other two in that no storage vault is required. Each canister would be sealed in a two-inch thick steel cask which would be surrounded by a 38-inch thick concrete shield to provide radiation protection. A six-inch air space between the concrete and the steel cask would allow cooling air to circulate, which would keep the temperature of the cask to about 120° C. The massive concrete storage units would be kept outside, and by the year 2010 the entire storage area could occupy about 1,100 acres of land.

The advantages of this design are, again, that no mechanical cooling system is required, but the disadvantages are the large amounts of land needed and the fact that in the unlikely event of a container rupture some radioactive wastes would be lost to the atmosphere.

The three potential designs are now being looked at by various groups, including a committee of the National Academy of Sciences, and when a final choice is made, the AEC will publish another, more comprehensive environmental impact statement.

The choice of design will, to some extent, also affect the choice of possible site. The AEC has indicated that it is now considering potential sites at the Hanford Reservation in Washington State, the National Reactor Testing Station in Idaho, and the nuclear test site in Nevada. Each of those would be suitable for the two air-cooled designs, but because of the shortage of water the Nevada site would be unsuitable for the water basin design.

In the meantime, the AEC has also been examining a few so-called unconventional waste disposal methods, such as burial in polar ice sheets, placement on the seabed, discharge into volcanoes, or loading the wastes on a rocket and firing them into the Sun. A study conducted by Battelle laboratories has concluded that none of those concepts presents a viable alternative to burial in a stable geological formation such as bedded salt.

Even if the storage facilities will, as the AEC claims, pose little radiation risk to people living close to them—even in the event of the "maximum credible accident" of a canister rupture during storage or handling—the plans outlined in the environmental impact statement are sure to meet with considerable opposition.

For one thing, large numbers of shipments of waste canisters will be required—about 600 a year by 2010—to transfer wastes from reprocessing plants to the storage site. It was, in fact, this aspect which raised most of the opposition in Kansas to the proposed repository at Lyons, and there is no reason to expect that attitudes will change.

But the most powerful argument, which is already being resurrected, is that since there is absolutely no guarantee that a permanent disposal site will be found, the entire nuclear power programme represents a Faustian bargain in which relatively short-term energy benefits have been traded for a very long-term pollution problem. ☐

Court ruling has its dangers

by Colin Norman, Washington

THANKS to a little-noticed ruling of the Federal Appeals Court in Washington DC, research proposals contained in any grant application submitted to the federal government must be disclosed to anybody who asks to see them—including another scientist working in the same field.

Since grant applications, particularly those submitted to the National Institutes of Health, usually contain a fairly detailed description of the proposed experiments and how they will be carried out, several scientists reacted with some alarm when informed of the court decision last week. And, within NIH itself, there is a general feeling that the ruling will affect the way in which grant applications are written and reviewed.

Brought by a public interest law group called the Washington Research Project Inc., the court case itself involved eleven specific grant applications for research into the effects of psychotropic drugs on children with learning disabilities.

A lower court judge ruled in November last year that the Freedom of Information Act (FIA) requires that not only the grant applications but also the reports of peer-review groups which rate them according to their scientific merit, should be made public. The Appeals Court decided last week, however, that while the grant applications should, indeed, be publicly available, the confidentiality of peer-review reports should be preserved.

A fight is now likely to ensue over exactly how the ruling should be interpreted. Although a final determination had not then been reached, government lawyers suggested last week that since the eleven grant applications in question had all been approved and funded by the National Institute of Mental Health, the decision probably does not apply to applications which are still pending. But a lawyer for the Washington Research Project, citing language in the decision which refers to "approved or pending" applications, argues that it does.

In any case, even if the ruling does apply only to approved grants, it would still cause a radical break from established NIH practice and hold some important implications.

First, there is the possibility of plagiarism of research ideas. According to the Appeal Court judge, the government lawyers took "pains to argue that biomedical researchers are really a mean-spirited lot who pursue self-interest as ruthlessly as the Barbary pirates did in their own chosen field". They argued, in fact, that a scientist would be able to get complete details of a competitor's experimental designs simply by asking NIH for them, conducting the experiments himself, and getting into print with the results before the hapless originator of the idea had been able to do so.

There are, however, rather more subtle ways in which the decision could influence the course of scientific research. It has been argued that one result will be that grant applicants will be less than explicit in giving details of their proposed experiments. Not only would that make the job of scientific review committees more difficult, but it could also favour those scientists who have an established track record.

Another possible consequence, cited by the Association of American Medical Colleges in a court brief, is that "pharmaceutical companies could periodically review NIH and NIMH files for the purpose of converting research findings to commercial use, thereby converting the government into a conduit of free research and methodologies for private gain". Furthermore, sinc the drug companies would also be made aware of what research the government is funding, they would be less inclined to invest in those areas, "thus discouraging independent biomedical research", the AAMC argues.

On the other side of the coin, however, it can be argued that taxpayers have a right to know exactly how the federal government is spending their money, and it would be contrary to the spirit of open government embodied in the Freedom of Information Act to keep research applications confidential.

Be that as it may, the government will probably appeal the case to the Supreme Court, although no decision has yet been made on that score. Alternatively, Congress could attach an amendment either to the Freedom of Information Act, or to other legislation such as appropriations bills, to preserve the confidentiality of grant applications. A move by Senator Robert Dole earlier this year to attach just such an amendment to the Freedom of Information Act was, however, turned back pending the court decision, and Congress is, in any case, unlikely to pass such a measure without first giving it some study at the committee level. □

Israeli jobs for Soviet scientists

from Nechemia Meyers, Rehovot

WILL Israel be able to absorb the 60,000 Jews a year, many of them engineers and scientists, who will reportedly be permitted to leave the U.S.S.R. under the terms of an unpublished agreement between the U.S. government and the Soviet government? To be sure, there is no shortage of jobs in Israel. The building trades, agriculture, industry and various services could absorb thousands of newcomers each year. However, positions are not so readily available for university graduates. Even now, according to Absorption Ministry Director General Menahem Sherman, 1,400 are without immediate prospects of a job.

No one knows how many scientists and engineers will be allowed out, but a large number could appear on the Israeli labour market in a relatively short period of time. Indeed, there are more Jewish scientists and engineers in the Soviet Union—an estimated 100,000—than in Israel, and Israel, of course, has an infinitely smaller economy in which to absorb them.

Israel's National Council for Research and Development has already established a special committee to approve Government allocations to new research units based primarily on immigrant scientists. Groups recently established with NCRD aid include one designed to foster the industrial utilisation of Negev Desert chemicals, another working on tools using radiation for inspection purposes and a third in the field of biomedical engineering.

Dr Vladimir (Ze'ev) Zaretskii (until 1971 a member of the Institute for the Chemistry of Natural Products of the U.S.S.R. Academy of Sciences in Moscow, and now in charge of the Weizmann Institute's Organic Mass Spectrometry Centre) believes that a large number of his former colleagues can be absorbed and help Israel's development at the same time.

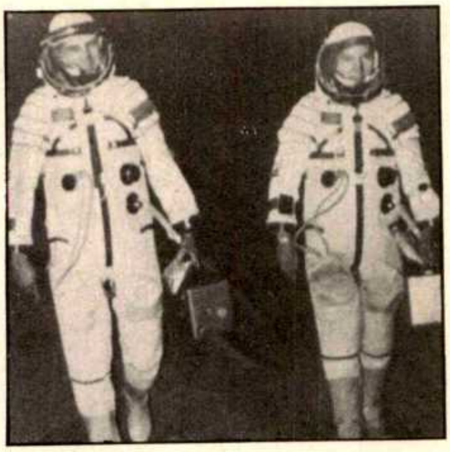
"Their place," he says, "is in industrial research. Thousands upon thousands of talented Jewish scientists and engineers work at centres of applied science. Their talents could be utilised by existing Israeli companies or by specially created new ones."

While welcoming the NCRD bodies, Dr Zaretskii believes an even closer link should be forged between industry on the one hand, and immigrant scientists and engineers on the other. He suggests partnerships that would bring together the research knowhow of the Russians with the business knowhow of Israelis and Americans. "People trained in the U.S.S.R. don't understand Western concepts of profit margins and marketing. But they do know how to work," Zaretskii says.

Certainly this isn't the best time for industrial expansion. Israel's resources are strained to the limit and beyond by defence needs. Moreover, it is becoming increasingly difficult to export goods at a time when world markets are shrinking and country after country is sliding into a recession. □

Soyuz-15: inside information

by John Gribbin



Soyuz–15 crew: could have docked

THE abortive flight of Soyuz-15 provided valuable tests of an automatic docking system intended for use by future "tanker" spacecraft. That at least is the inside story being put about at the Johnson Space Center by Major General Vladimir Shatalov, the Soviet cosmonaut chief (*Aviation Week & Space Technology*, September 16, 22; 1974).

Shatalov has been visiting the Apollo-Soyuz Test Project crew training sessions, and took advantage of the opportunity to give more details of the official Soviet view of Soyuz-15. Confirming that the spacecraft never made physical contact with the space station Salyut 3, he said that the automatic rendezvous system repeatedly took the spacecraft to within 30 to 50 m of the space station, but that the final rendezvous sequence failed each time.

According to Shatalov, docking using manual control would have been possible on any of these occasions. But that was not the purpose of the mission, and the Soviets preferred to spend the available time in repeated tests of the automatic docking system. Time was limited, apparently, because the Soyuz-15 mission was from the outset planned to last only two days, and was intended to land at night to test techniques. Even if docking had been achieved, it seems that the cosmonauts would have undocked and returned—after further docking tests—without entering the Salyut.

The official view is that the flight was a success because it tested the automatic docking system. This system will be needed in future developments of the manned space programme, when "tanker" derivatives of Soyuz will be used in an unmanned configuration to ferry fuel and other supplies up to space stations.

In the USA, there is some scepticism about Shatalov's outline of the Soyuz-15 mission. It is felt very unlikely that two men would have been sent to dock with Salyut-3 without any intention of their entering the space station, and it is also pointed out that the very first docking of two Soviet spacecraft involved unmanned vehicles, and was accomplished seven years ago. Although it seems unlikely that Soyuz difficulties of the kind experienced by Soyuz-15 are related to the joint programme, Senator William Proxmire has expressed concern and says that "present plans for a joint space mission in 1975 should be seriously re-examined in the light of the continuing difficulty in the Soviet program".

But perhaps his fears—and those of others—might be at least partly allayed by two other items of news made public last week. General Shatalov has publicly thanked his US counterpart, Brigadier General Thomas P. Stafford, and US astronauts for not revealing details of Soyuz-14 prior to the launch. It seems that the names of the cosmonauts and the date of launch were known to US astronauts training in the USSR last July, but that at the request of Soviet officials they said nothing to the press "in consideration of Soviet traditions and customs".

That, according to Shatalov, demonstrates the mutual trust needed to make the joint mission work. The other news, however, concerns more tangible preparations. Shatalov has now confirmed that at least one manned mission will be carried out to test Apollo-Soyuz hardware, about two months before the planned date of the joint mission, which is next July. This proving flight can only be carried out by the Soviet team, since NASA only has one Apollo/Saturn vehicle left, to use on the joint mission itself.

300 universities look to the future

from a Correspondent

THE General Assembly of CRE meets once every five years; often enough, it might be thought by those to whom the sight of one Vice-Chancellor is daunting enough, let alone 300. The theme of the Assembly at its meeting recently in Bologna was "The European Universities 1975–1985", and the Assembly divided itself into five groups to discuss particularly the University and the Changing Needs of Society, University Training, University Research, University Government, and the Financing of Universities.

After four half-day sessions the whole Assembly met to hear reports from the five discussion groups. With one or two honourable exceptions, the working papers which had been prepared before the Assembly could only be described as lead balloons, but a combination of good discussion in the groups and excellent work by the chairmen and rapporteurs led to useful and sensible final reports.

The report of the first study group (the University and the Changing Needs of Society) stressed particularly the difficulties that the universities have in responding to the needs of society when those needs are only imperfectly articulated in the present, to say nothing of needs in the future. This made it all the more important that universities, so far as they can, should give not only academic advice to students but should help them with the role they were later to play in society. Since migration in the field of academic professions is growing, an international understanding of the equivalence of diplomas and degrees needed to be established, although the Assembly was very much aware of the danger for the "Third World" of losing some of their best people to the developed countries. While the university should be responsive to society's demands, it should keep constantly before itself its special role as "the place where the most complex and profound work can be done". Perhaps the surprise of this session (at least to Western Europeans) was the "points" system of university entrance practised in Eastern European countries and described by the Rector of Warsaw University, where points could be granted not only to first-generation children of peasant origin but also to winners of sporting events. In Poland and Czechoslovakia, for example, winners of events in national Olympic games are exempted from university entrance examinations.

There was considerable disquiet expressed in the discussion group on University Research about the squeeze on funds for research as financial pressure on universities increased, and how important it was to explain to the public and politicians the essential role of research in universities.

So far as international research projects were concerned, although these were generally to be welcomed, they could be successful only if there was a genuine scholarly need for them. In some cases (and CERN was cited as an example) these projects had been outstandingly successful, but this had not been uniformly the case. Sponsored research was discussed at some length and with enthusiasm. The group felt, however, that there were two basic conditions which must be met by such research: the first was that it must be stipulated that the scientific content of such projects should always be published; the second was that sponsored research should not interfere with the general research policy and the teaching duties of the university.

In the discussions of the financing of universities it was emphasised that, although universities needed to know basic data—such as staff/student ratios, number of square metres/student—for their proper management, these data had to be looked at with an expert eye if they were to be meaningful. There were no strong views about the relative merits of grants or loans for the support of students, but the group was strongly against the notion that undergraduate students, at least, should be salaried and regarded as clients. It was necessary for the university to have strong leadership if it wanted a proper system for setting priorities and wanted to retain proper autonomy.

So, all in all, was their journey really necessary? Most decidedly so, most of the delegates felt at the end. The conference had emphasised once more the strong links which unite European scholarship and the necessity of preserving and nurturing those links.

correspondence

Adding amino acids

SIR,—The issues that Drs Miller and Payne raise (*Nature*, **251**, 176) may be summarised as follows:

1. No example is available of a field trial that shows measurable benefit of amino acid addition;
2. I confuse the problem of meeting physiological needs for protein with that of aesthetic demands;
3. Methionine, not lysine, is limiting in most diets, including cereal diets;
4. Talk of amino acid fortification constitutes a diversion from the main problem, poverty of large masses;
5. The only solution to the problem of malnutrition is to raise the standard of living and improve traditional agriculture.

It is true that examples of completed field trials are lacking. As I stated, (*Nature*, **248**, 643) some are underway; others might well be started in which non-protein calories (such as cassava, sago, and plaintain, or oil) could be added to a predominantly cereal diet with added amino acids to determine how to extend the range of options for obtaining food energy balanced with protein.

I do not confuse physiological needs and aesthetic demands and stated so clearly in the section entitled "No Substitute for Aesthetics".

That methionine is limiting in cereal diets has been disputed. Bressani and Elias[1] pointed out that only when legume contributes more than 50% of the protein in a cereal-legume mixture is methionine limiting; at that level the diet is not protein-deficient.

The world protein food problem has had an extraordinary history: it is the only major world problem that has been discovered and solved, all within a period of about 25 years, and simply by changing standards. More to the point would be statistics on the prevalence of protein–calorie malnutrition of the kind that indicates a particular deficiency of protein—the kind that was called "kwashiorkor". Has this diminished along with the disappearance of the protein problem? And if, indeed, there is an increase of protein–calorie malnutrition of all types, then does this not emphasise even more the need to provide more food energy and point out the consequences of the protein–calorie trade-off? That amino acid fortification does not reach all elements of a population or that it does not provide additional aesthetics in food is of secondary consequence: the critical point is that additional ways of adding protein make it possible to divert resources to more food energy, or special baby foods, or more pleasing foods.

The world food problem is an extraordinarily complex problem of massive poverty exacerbated by increasing populations and shrinking resources. What Drs Miller and Payne have to offer has been tried again and again, and has been shown inadequate. Only a total world attack can deal with this problem: affluent nations will need to distribute more of their wealth to the poor nations at the cost of a reduction of some aspect of their own quality of life; the poor nations will need to develop policies that reduce the ratio of populations to resources. Ultimately, decisions to act involve moral considerations. But such decisions involve many other factors, among them the availability of solutions and the multiplicity of options. Is it right to deny poor countries the use of technologies that could increase their opportunities to solve problems as they cope with the basic problem of poverty?

AARON M. ALTSCHUL

Washington DC.

[1] Bressani, R., and Elias, L. G., in *New Protein Foods* (edit. by Altschul, A. M.), 282 (Academic Press, 1974).

Publish it not

SIR,—I am profoundly shocked by the leading article in the issue of *Nature* of September 6. My dismay arises from the content of the fourth and fifth paragraphs. Firstly it seems to me to betray a surprising ignorance of the issue under discussion to draw an analogy with weather modification or shocking experimental animals. In both cases we know the consequences to be localised and if a scientist in Idaho does modify the weather, this has no consequences in India or Iceland. Climatic modification is of course a different issue. But with genome fusions, there is no reason to believe that a blunder might not have consequences which would affect very large numbers of people over a very wide area of the globe.

The second issue relates to your concept of "pushing (papers) into the underground". Certainly there are undergrounds: one is concerned with military research and the other industrial research. But it seems reasonably certain (military matters apart) that the people concerned in this particular field are ones who desire public recognition for their work and who would be discouraged very quickly if they saw that their findings would not lead to the glories of a mention in the Citation Index.

Yours faithfully,
D. W. EWER

London.

Family favourites

SIR,—On page 98 and 99 of vol. 251, there are 3 consecutive contributors named Davies.

Statistical analysis shows that the probability of this arising on the basis of chance is infinitesimally small, so it can only be nepotism!

A. G. GORDON

London.

news and views

Digesting DNA and chromatin

ALTHOUGH the basic double-helical structure of DNA has been known for many years its sequence arrangements and spatial organisation in chromatin have remained poorly understood The discovery of restriction enzymes, which cleave double-stranded DNA at specific target sites, has given a much needed boost to sequence analysis of DNA Recently some interesting results concerning the organisation of satellite DNAs have been obtained using the restriction enzyme EcoRI, which cleaves DNA at the sequence GAATTC The size of DNA fragments generated by the enzyme reflects the separation of target sites and so a limited base sequence complexity, as exists in satellite DNAs, would tend to result in infrequent cleavage

Botchan *et al* (*Cold Spring Harb Symp quant Biol*, **38**, 383, 1974) found that mouse satellite DNA could be separated from the rest of the DNA by cleavage with EcoRI The satellite DNA formed a slow migrating band on electrophoresis whose fragments ranged in molecular weight from 4×10^6 to 20×10^6, indicating that it is composed of inexact repeating units In contrast, Botchan (in this issue of Nature, page 288) finds that the same enzyme cleaves bovine satellite I DNA into highly repetitious units of 1,400 base pairs Renaturation kinetics indicate that these units are internally repetitious He proposes that two stages were involved in the evolution of satellite I DNA The first stage involved small duplications, indicated by the renaturation rate, to yield the 1,400 base pair unit, and the second stage involved the production of large tandem repeats of this unit Such analysis of other satellites should give a clearer picture of how satellite DNAs are organised and perhaps confirm this hypothesis Mouse satellite DNA, if further analysis does not reveal some higher order of repeating units, may be a case where subsequent divergence has obscured the homogeneity of the tandem arrays

On another level of DNA organisation recent work points to a highly repetitive association of protein with DNA in chromatin Olins and Olins (*Science*, **183**, 330, 1974) reported linear arrays of chromatin particles, which they termed ν bodies, each about 70 Å in diameter They calculated that each ν particle could contain at least one, possibly two, molecule of each of the five main histones and a stretch of DNA of molecular weight between 80,000 and 160,000 Sahasrabuddhe and Van Holde (*J biol Chem*, **249**, 152, 1974) isolated micrococcal nuclease resistant particles constituting 50% of the total DNA from calf thymus chromatin with sedimentation coefficients of between 10S and 12S These particles contained approximately 100 base pairs and had an estimated diameter of about 80 Å Similarly Clark and Felsenfeld (*Biochemistry*, **13**, 3622, 1974) have found that, on digestion with micrococcal nuclease, 50% of the DNA in chromatin remains as protected areas containing roughly 100 base pairs Hewish and Burgoyne (*Biochem biophys Res Commun*, **52**, 504; 1973), on the basis of nuclease digestion studies using an endogenous Ca^{2+}–Mg^{2+} dependent endonuclease of rat liver chromatin, have proposed a simple, basic, repeating structure for chromatin Kornberg (*Science*, **184**, 868, 1974) has elaborated this into a theory of chromatin structure based on a repeating unit containing two each of the main histones (excluding F1 histone) and about 200 base pairs of DNA forming a flexibly jointed chain Support for this theory now comes from the work of Noll (*Nature*, **251**, 249, 1974) who finds that using both the endogenous Ca^{2+}–Mg^{2+} dependent endonuclease and micrococcal nuclease approximately 85% of rat liver chromatin can be digested into 11S fragments containing about 200 base pairs Further digestion, however, causes a breakdown of DNA in these fragments The isolated fragments contain all five major histones and some nonhistone proteins

Thus evidence is accumulating for a regular spatial arrangement of proteins in chromatin, the proteins protecting stretches of DNA from nuclease attack Further studies may show a simple relationship between the 200 and 100 base pair fragments, for example a totally protected 100 base pair unit associated with 100 base pairs which are partially protected T BARRETT

Termination and the juggernaut polymerase

GENE expression seems in general to be controlled at the initiation of transcription An alternative mechanism operates, however, during the development of some phages Initially 'early' genes are transcribed by a host (bacterial) polymerase which is halted by termination signals Subsequently an anti-termination protein is synthesised to allow transcription beyond these signals The mechanisms of termination and of anti-termination have not been well defined and their role within the uninfected bacterium remains particularly unclear Adya, Gottesman and De Crombrugghe (*Proc natn Acad Sci U S A*, **71**, 2534–2538, 1974) now suggest that the mode of termination may be dictated by events taking place at initiation, their work with polar mutants also reveals a new aspect of the connection between translation and transcription

Expression of the galactose operon of *Escherichia coli* is different when caused by readthrough from a prophage instead of the normal initiation at a galactose promotor When prophage lambda is induced, constitutive ('escape') synthesis of the nearby galactose enzymes ensues, for two reasons (1) replication of the galactose structural genes together with the prophage increases the number of operators so that insufficient repressor is present in the cell to prevent transcription, (2) transcription is initiated at the p_l (*sex*) promotor and proceeds from the prophage, through the intervening bacterial genes, into the galactose operon Certain mutations permit only the second mechanism to operate, by preventing the replication and the excision from the chromosome of the prophage

Adya *et al* used an increase in temperature from 32° C to 41° C to induce prophage bearing such mutations They found a seven-fold increase in the specific activity of galactokinase, the enzyme coded by *galK*, the last of the three structural genes of the galactose operon Since the addition of fucose, an inducer of the galactose operon, causes a further increase in galactokinase synthesis, apparently to the same extent as usual, expression by induction and expression by readthrough seem to be additive This suggests that they take place by different mechanisms, and implies that galactose repressor can inhibit only transcription that initiates at the adjacent galactose promotor, and does not act simply by blocking progress of polymerase into the structural genes This is consistent with the observation of Nakanishi *et al* (*J biol Chem*, **248**, 5937–5942, 1973) that repressor and polymerase seem to compete *in vitro* for galactose DNA and also with the dual control noticed in the galactose operon—there appear to be two operator/promotor sites, whose expression is additive but each of whose repression does not prevent expression from the other (see Hua and Markovitz, *Proc natn Acad Sci U S A*, **71**, 507–511, 1974)

Termination of transcription in bacterial DNA is not well defined, but sites in λ DNA have been mapped at which rho factor causes RNA polymerase to cease transcription *in vitro* As the λ map below shows, one site, (t_l), terminates leftward early transcription from p_l, another, (t_r), terminates rightward early transcription from p

Since only the early genes *N* and *tof* are expressed upon induction *in vivo* prior to synthesis of N protein, the same mechanism presumably prevails in the cell It is the synthesis of N protein which allows transcription to continue to the left past t_l and to the right past t_r

galK galT galE galO/P *exo cIII* t_l *N* p_l/o_l

p_r/o_r *tof* t_r *O P Q*

The accepted interpretation of these results has been to suppose that N protein acts at the sites t_l and t_r where transcription terminates But another interpretation is supported by the results of Adya *et al* Readthrough transcription of the galactose genes depends upon N protein since with an N^- prophage there is no escape synthesis of galactokinase—presumably transcription halts at the t_l site in the absence of N protein That the readthrough transcription supported by N has started at p_l is shown by the reduced readthrough synthesis of galactokinase found when a prophage bearing the *sex* mutation in p_l is induced The 12 min delay between prophage induction and galactokinase synthesis also is consistent with the idea that RNA polymerase must traverse the long distance between p_l and the *gal* genes, and the RNA made by readthrough is much larger than the usual 12–22S mRNA produced by fucose induction

Mutants in which foreign DNA has been inserted into the galactose operon constitute one of the few cases in which rho terminates transcription *in vitro*, De Crombugghe *et al* (*Nature new Biol*, **241**, 260–264, 1973) suggested that the polar effects of these mutations *in vivo* result from termination caused by rho and not by nonsense codons Polar mutations inactivate the gene in which they are located but also reduce the extent of expression of subsequent genes in the operon But when transcribed by N-dependent readthrough from the p_l promotor, the polarity of such an insertion in *galE* is suppressed, galactokinase can be synthesised Even more surprising is that the polarity of an entirely different type of insertion (not rho-sensitive) and a polar ochre nonsense mutation also are suppressed under readthrough conditions

The ability of polymerases that initiate transcription at p_l to pass subsequent rho-dependent termination sites thus contrasts with the inability to do so of polymerases that initiate at the galactose promotor The N protein responsible for anti-termination cannot, therefore, simply act at the termination sites at which its effect is exerted Rather does anti-termination depend upon where the polymerase initiates transcription Since phage 21 (which differs in the *N* gene from phage λ) canot complement λN^- mutants to allow readthrough, the site of action of N must be located in the region that is non-homologous between the phages This would be consistent with the idea that N acts at the p_l site, attaching to polymerase there, and turning it into what Adya *et al* describe as a "juggernaut" that ignores all rho-dependent signals for termination Thus only polymerases initiating at the site where N attaches to the enzyme can acquire the juggernaut capacity, all other polymerases terminate normally at rho-dependent sites The polymerase is seen as an enzyme to which other proteins may attach, in this case conferring anti-termination ability, perhaps in other cases changing the specificity of initiation

The effect on transcription of the initiation site is also seen by comparing the tryptophan operon located on the *E coli* chromosome with that translocated to a position to the left of gene *N* of phage λ when readthrough can occur Segawa and Imamoto (*J molec Biol*, **87**, No 4, 1974) show that nonsense mutations in the *trpE* (first) gene cause production of truncated messengers when transcription initiates at the tryptophan promotor, but long messengers continue to be synthesised when transcription instead takes place by readthrough from p_l Results showing that polarity of nonsense mutations is suppressed by readthrough transcription are cited from two other groups as in press

Poly (C) in viruses

Porter, Fellner and Carey (*Nature*, **248**, 675–678, 1974) recently showed that encephalomyocarditis (EMC) virus RNA contains a poly (C) tract which is about 100 nucleotides long and contains 80–90 C residues The authors argued that the poly (C) tract is probably not translated into protein but instead suggested that it may be involved in viral replication, possibly as a replicase binding site On page 342 of this issue of *Nature*, Brown, Newman, Stott, Porter, Frisby, Newton, Carey and Fellner report a similar analysis of several other picornaviruses, including some of economic and medical importance, in the hope that an understanding of the universality of poly (C) might give some clue as to its function In addition, they suggest that if poly (C) should prove to be virus specific then it may be possible to exploit the presence of this unusual structural feature in the design of specific antiviral reagents

Picornaviruses can be divided by physiochemical methods into five subgroups enteroviruses (for example polio, swine vesicular disease), cardioviruses (such as EMC, Maus Elberfeld (ME)), foot and mouth disease viruses (FMDV) and human and equine rhinoviruses Poly (C) tracts were detected in all the cardioviruses and FMD viruses examined but not in six different enteroviruses, three human rhinoviruses, nor in the togavirus Semliki Forest Virus The presence of poly (C) is therefore genus specific, and Brown *et al* point out that their oligonucleotide 'fingerprinting' data supports the subgrouping of picornaviruses mentioned above

The length of the poly (C) tract also varies, not only between viral subgroups but surprisingly between different strains and different subtypes of one serotype For example the cardioviruses EMC and ME have poly (C) tracts of 100 and 150 nucleotides respectively, and FMDV strains A61 and CGC, 150 and 200 nucleotides respectively

The location of the poly (C) within the viral RNA molecules is not yet certain but it is possible that the longest fragments originate from the 3′ terminus of the viral RNA If this proves to be the case then it is not surprising that the enteroviruses do not contain poly (C) since they contain the more familiar poly (A) tail in this position At present the function of poly (C), like that of poly (A), is unknown, it will be of interest to know whether similar structures are found in other, more exotic viral RNAs, or in host cell mRNA or HnRNA

Alan E Smith

(Zalkin, Yanofsky and Squires, *J biol Chem* , Franklin, *J molec Biol*)

The ability of a juggernaut polymerase to suppress polar nonsense mutants implies that all polar effects may be mediated at the level of transcription This resolves a conflict of some years standing as to how premature termination of protein synthesis may affect the availability of messenger for subsequent genes One view has been that dissociation of ribosomes within (but not at the end of) a gene makes the subsequent messenger regions susceptible to degradation, an alternative was to suppose that termination of transcription within a gene causes transcription to halt, although it has always been difficult to visualise molecular models for this relationship (see Lewin, *Nature new Biol*, **232**, 161–162, 1971) In view of the action of N as an anti-terminator, these new results support the idea that premature translational termination may provoke a rho-dependent transcriptional termination that can be antagonised by the anti-terminator action of N

Adhya *et al* propose that termination of translation in a gene allows rho-dependent termination of transcription to take place at the end of the gene by a normal polymerase, although a juggernaut polymerase may of course read straight through, suppressing the polarity of the mutation This implies that a rho-dependent termination site is present at the end of each gene in which polar mutations may occur but is rarely recognised in the usual course of events The termination signal at the end of an operon presumably differs from any internal signals and is recognised by all polymerases

Benjamin Lewin

Do pulsars make supernovae ?

from James Pringle

The brilliant explosion of a supernova heralds the demise of a highly-evolved, massive star When stars in the mass range 3–8 solar masses ($M_\odot$) have exhausted the hydrogen and helium in their central regions, they all evolve to red-giant configurations of very similar structure They have a dense core of about $1 M_\odot$, surrounded by a zone containing shells of burning helium and hydrogen, the whole of which is engulfed in a highly extended low-density envelope about a thousand times larger than the Sun

The details of what happens next are still uncertain It is known that some kind of explosion is triggered off, releasing in all about 10^{50} erg The outer layers of the star are blown off at speeds of some 8,000 km s^{-1}, gathering up the interstellar gas in their path and later becoming the wisps of gas which are known as 'supernova remnants' and of which the Crab Nebula is the most oft quoted example Eventually, the 'remnant' itself disperses to become part of the ambient interstellar medium which, in its turn, condenses to form stars It is precisely because a supernova explosion spews heavy elements from the heart of the dead star into the interstellar medium, that planets too can form and life as it is known can exist

Several specific mechanisms for triggering the initial explosion have been advanced—for example, the suggestions by Fowler and Hoyle that the core collapses caused by the photodisintegration of iron or that the explosive onset of carbon burning disintegrates the whole star Recent evolutionary calculations strongly indicate that collapse of the core in a highly evolved star, rather than its total disruption, is possible, at least for stars of 3–8 $M_\odot$ The collapsed core forms a 'neutron star'—a highly compact object with a typical density of 10^{16} g cm^{-3} This density is so great that the star's original magnetic field is compressed to a value around 10^{12} G and any rotation the core had before collapse is amplified until the newborn neutron star has a rotation period of a few milliseconds Thus a sizeable fraction of the gravitational energy released by the collapse of the core is converted into and stored as the rotational energy of the compact object The combination of rapid rotation and strong magnetic field causes most of the kinetic energy thus stored to be radiated away within a few thousand years, although the slow radiation of the residual rotational energy can be observed for much longer in the radio band as the regular bleeps from a pulsar These ideas are confirmed by the presence of two very short period, and presumably very young, pulsars in supernova remnants —the nebular remnants around the older pulsars would presumably have dissipated by now

Thus, the implosion of the stellar core is required, first, to release enough momentum to disperse the outer layers of the star at high velocity and, second, to release enough energy to account for the visual light curve Light curves have been caculated according to several different theories, but, in general, the first requirement is a problem if the energy is released on too short a timescale Two recent articles, one by Ostriker and Gunn (*Astrophys J Lett*, **164**, L95, 1971) and the other by Bodenheimer and Ostriker (*Astrophys J*, **191**, 465, 1974) suggest that the pulsar at the centre of the supernova can make a substantial contribution to the energy of the outburst Rather than having to release all the energy in one initial explosion, the authors argue that the power continuously radiated by the newborn pulsar is sufficient to assist in propelling the outer stellar layers up to high velocities They perform detailed calulations, assuming that the main energy source in a supernova is the dipole radiation from the central pulsar If it is intense enough, the radiation hollows out a cavity in the centre of the star and then expels the outer envelope at high speed As this shell of material moves outward, its density decreases rapidly and its transparency increases accordingly The peak of the light curve occurs when the timescale for the expansion of the envelope is approximately equal to the timescale on which photons can diffuse outwards through it The authors obtain good agreement with the observations of 'Type II' supernova light curves They comment on other effects—such as the possible presence of a dust shell around the pre-supernova star—which add complications and could improve agreement They predict that about 3–4 months after the light curve maximum ultraviolet and X radiation from the central cavity should become visible

Many a marmot

from our Animal Ecology Correspondent

All populations have a tendency to overproduce and, if they are not held in check, to damage the environment upon which they depend for existence Some ecologists believe that control is enforced only by the actions of extra-population forces, such as the climate Others hold that mechanisms which are dependent on the density of the population and which modify their effect according to the state of the population are at work In proposing this system of control, Nicholson stated that the only perfect density-dependent regulatory factor is intraspecific competition, all other factors play less direct, though strongly influential parts (*J Anim Ecol*, **2**, 132, 1933) Wynne-Edwards endorsed Nicholson's views and drew attention to the part played by social behaviour in the dispersion, and hence regulation, of animal populations (*Animal Dispersion in Relation to Social Behaviour*, Oliver and Boyd, Edinburgh, 1962) He stressed that intraspecific competition, be it for a territory, individual range, rank position in a hierarchy or the like, is never directed at the environmental resource in short supply but at a substitute The winner of a substitute goal gains exclusive rights to some primary environmental resource

If social behaviour really does play such an important part in the prevention of overpopulation (by allowing only a fixed number of goals to be open to competition) one might expect to observe changes where the environment embraces both harsh and kind habitats To investigate this the North American marmot offers as good a study animal as has yet ben found The species are morphologically very alike and occupy a huge range of habitats from the mountain tops above the tree line (Olympic marmot) through the upper forests to the valleys (yellow-bellied marmot) to the plains at sea level (woodchuck) A most interesting gradation in social behaviour has been reported by Barash which, if the interpretations are correct, shows clearly that the quality of social behaviour is correlated with the harshness of the environment (*Science*, **185**, 415, 1974)

Woodchucks living in fields and meadows at low elevation experience a growing season, when their food is rich and abundant, of about 150 days They are solitary and aggressive The male–female association last only as long as the brief courtship and mating themselves Females breed every year and their offspring disperse before the winter The yellow-bellied marmot lives at elevations where the growing season may last less than 100 days It is less solitary than the woodchuck but still maintains home areas They show a greeting behaviour—a pattern never seen in woodchucks—and greet their neighbours two or three times daily The Olympic marmot is restricted to the treeless alpine meadows of Olympic National Park, Washington, where the growing season may be as short as 40 days These are highly gregarious animals which live in well organised colonies of adults, 2-year-olds, yearlings and infants They do not protect home areas and wander about the colony without fear of attack Their level of aggression is low and in consequence greet one another about ten times as frequently as their yellow-bellied cousins Females breed every other year and youngsters do not disperse until they are 2 years old By the time they reach this age they weigh 70% of the adult weight In comparison woodchucks (weighing 80% of adult weight) disperse before their first winter

The important thing for a species is to leave the maximum number of surviving offspring that the habitat will carry Because of the varying durations of growing seasons encountered by marmots the optimum age for dispersal varies between a few months and 2 years Barash demonstrates that the environment dictates when dispersal takes place through the effect of growth rate on aggressiveness If over-winter mortality has been high and the population needs replenishing, the level of aggression is a little lower and a higher proportion of the dispersers remain, and *vice versa*—a very clear example of adaptive social behaviour

Several factors may complicate Barash's simple interpretation—for example, female Olympic marmots breed once in 2 years while the other species bred annually The reproductive physiology and feeding ecology of a species play a fundamental part in moulding the shape of the behavioural fine tuning Barash's work demonstrates beautifully that the barrier between ethology and ecology is more imagined than real

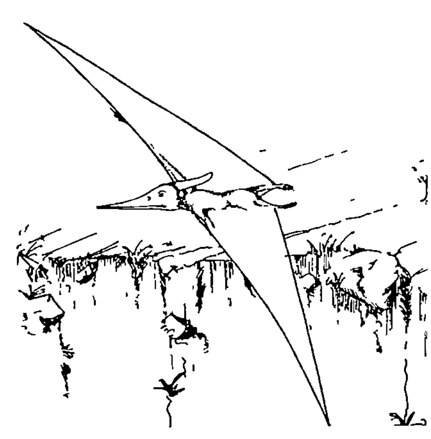
Prehistoric gliders

from a Correspondent

IN a study of the biomechanics of *Pteranodon*, Bramwell and Whitfield discuss the structure and aerodynamics of the largest flying animal to have existed As *Pteranodon* represented the end product of pterosaur evolution it was likely to show a high degree of structural and aerodynamic refinement and this has been largely confirmed *within this study* (*Phil Trans R Soc*, **B267**, 503–592, 1974)

Any such study as this is based upon conjecture—indeed herein lies one of its fascinations—but the authors have minimised uncertainty in this respect by utilising an interdisciplinary approach, combining both engineering and palaeontology to formulate conclusions The application of engineering to an extinct animal is obviously extremely difficult, particularly as parts of the fossil animal were missing The authors have, however, used the best data available and where their conclusions are open to doubt this in no way infers a criticism of this study, but of the lack of information particularly within the field of low speed aerodynamics

From available fossil and estimated sizes the strength of the bones in the wing spars has been calculated The general factor of safety is approximately 5 which is a reasonable value for a flying animal with the assumed flight activities of *Pteranodon* The most surprising feature of the structure is a factor of safety of only 2 for the humerus It is possible that the assumed strength values for this bone were incorrect and the authors have considered this as one possibility by suggesting that a stronger, more brittle bone material was practical They also put forward a more feasible explanation in that part of the loads could be taken by some of the flight muscles being inserted well out along the humerus



Bramwell and Whitfield's interpretation of how *Pteranodon* soared in the hill lift caused by a cliff

From tests in a wind tunnel Bramwell and Whitfield provide a new explanation of the large crest on the animal's head This structure had previously been thought to have the effect of a rudder or steering device but these authors clearly show that it was a weight-saving device, by balancing the aerodynamic loads on the beak it could reduce the required neck muscles and thereby save weight

The aerodynamic characteristics of the wing were based on the use of data for the Gottingen 417a curved plate as being the nearest to the *Pteranodon* wing section There is a discrepancy between the drag values of the Gottingen 417a aerofoil section as presented by Schmitz and that presented in Figure 44 by Bramwell and Whitfield, but it is obvious that these authors have used the data from Schmitz for all their performance estimations

It is unfortunate that there are no more recent data than those for the 417a section nor any more appropriate aerofoil section on which to base the estimations of *Pteranodon*'s performance From a polar curve of *Pteranodon* the best flying speed was about $8\ \mathrm{m\ s^{-1}}$ with a minimum sinking speed of $0\ 43\ \mathrm{m\ s^{-1}}$ The highest useful flying speed was about $14\ \mathrm{m\ s^{-1}}$ These estimates of performance are realistic as any small discrepancies in the assumed wing drag characteristics are unlikely to have a profound effect on the overall lift/drag ratio

A comparison between the performances of *Pteranodon*, an albatross and a modern high performance glider shows that *Pteranodon* was a very efficient glider with a sinking speed of about half that of the other two From estimates of power required it seems that *Pteranodon* was also just capable of powered flight for short durations

Bramwell and Whitfield conclude that *Pteranodon* was primarily a glider that utilised sea thermals for lift while searching for food Although dynamic soaring is used by the albatross to sustain flight over the sea, the authors show that *Pteranodon* was unlikely to utilise this form of lift because of its low flying speed and the critical conditions for the required wind gradient

Heat flow in a metamorphic crust

from Peter J Smith

IN investigations which attempt to relate the Earth's heat flow to crustal structure and composition, it has been usual to view the crust in terms of homogeneous igneous rocks Thus, for example, when Roy *et al* (*Earth planet Sci Lett*, **5**, 1, 1968) demonstrated the correlation between geothermal flux and surface heat production, they made it clear that their measurements and analysis applied specifically to intrusive granitic bodies But as Smithson and Decker (*Earth planet Sci Lett*, **22**, 215, 1974) point out, although crustal models involving homogeneous igneous rocks have the merit of simplicity they may be a poor general representation of the real Earth Fifty years ago, Clarke and Washington (*Prof Pap US geol Surv*, **127**; 1924) could plausibly argue that 95% of crustal rocks are igneous, but modern work is more likely to stress the importance, and even the predominance, of metamorphic rocks with characteristic heterogeneities

Evidence for the modern view is extensive The Wind River Mountains of Wyoming, for example, are a broad anticlinal uplift with a core of Precambrian rocks largely comprising migmatite Borehole data suggest that in places such rock is at least 4 km thick, in addition to which denser metasedimentary rocks such as mica schists, garnet schists and metagraywacke may be found at structurally higher levels Again, a profile through the Ivrea zone of the Alps has been interpreted in terms of an upper crust comprising metamorphic rocks such as mica schist, a middle crust which is granitic in the form of migmatite and granitic gneiss, and a lower crust consisting of dehydrated mafic metamorphic rocks and diorites Migmatites underlying other metamorphic rock types are also found in Canada, east Greenland, southern Norway and central Europe Indeed, Barth and Reitan (in *The Precambrian*, Interscience, 1963) have estimated that as much as 93% of the exposed southern Norwegian Precambrian may be metamorphic—a figure which corresponds well with Wilson's picture (in the same volume) of the Canadian Shield as a region of metamorphic rocks "in which areas of granite and other igneous rocks are interspersed"

But where does all this (and much more) leave the analysis of terrestrial heat flow in terms of simple igneous and homogeneous structures? The answer is, of course, a rather poor approximation to the truth, but an approximation which can nevertheless be built on to give a picture closer to reality Smithson and Decker build by proposing the following three-zone model of stable continental crust

- The upper zone consists largely of metamorphic rocks of intermediate composition with a mean density in the range 2 75–2 80 g cm^{-3} and seismic P wave velocities ranging from 6 2 to 6 5 km s^{-1} Geological relationships and gravity interpretations suggest that this surface zone could be 5–12 km thick, although Smithson and Decker use a thickness of 8 km The metamorphic rocks actually comprise mica schists, metagraywackes (biotite gneiss and quartzo-feldspathic gneiss), greenstones and amphibolites According to Shaw (*Geochim cosmochim Acta*, **31**, 1111, 1961), the mean heat production within the Canadian Shield is 4 heat generation units (h g u), and it is known that intrusive granites generally have a higher heat production The intervening metamorphic rocks of the upper zone must therefore have a heat production lower than 4 h g u The estimate made by Smithson and Decker is 3 h g u, based on a mixture of mica schists, metagraywacke and mafic rocks
- The middle zone is also 8 km thick and comprises rocks which are less dense and more granitic than those of the zone above This is the migmatite zone and consists largely of granitic gneiss and augen gneiss intermixed and interlayed with biotite-rich gneiss and amphibolite lenses The mean density of this very heterogeneous region is about 2 72 g cm^{-3}, P wave velocities lie in the range 6 0–6 3 km s^{-1} and the highly variable heat production has a probable average of about 5 h g u
- The lower zone, 18 km thick, is composed of dense rocks (2 80–3 00 g cm^{-3}) with high seismic velocities (6 5–7 0 km s^{-1}) Both field and laboratory studies show that at likely pressures and temperatures in the lower crust mafic rocks would be recrystallised as metamorphic rocks in the amphibolite, granulite or eclogite facies, although the last of these is unlikely Smithson and Decker argue that granulite facies rocks actually comprise the bulk of the lower crust, although they expect pockets of amphibolite to coexist with them Heat production is estimated to be 0 5–5 h g u

Having proposed this more "realistic" model, however, Smithson and Decker are at pains to emphasise that the zones should not be interpreted as discrete layers The zones are heterogeneous and grade into each other irregularly, so that even the quoted thicknesses cannot be regarded as universal Average values tend to conceal considerable diversity in both the horizontal and vertical directions Thus the model is a generalisation which may apply in the specific form given only in relatively few localities

Notwithstanding these restrictions, if the surface heat flow for stable continental areas is taken to be 1 2 heat flow units (h f u) in accordance with the suggestion from Blackwell (in *The Structure and Physical Properties of the Earth's Crust*, American Geophysical Union, 1971), the model predicts a heat flow contribution of 0 3–0 5 h f u from the upper mantle The upper limit of this range then leads to temperatures of 130° C at a depth of 8 km, 407° C for the Moho at 34 km, and 538° C at 54 km in the upper mantle—temperatures which are comparable with, but slightly lower than, those obtained by Blackwell from other models The controversial point to arise here is the claim by Smithson and Decker that such low temperatures rule out the possibility of a universal seismic low-velocity zone produced in the crust by thermal effects A low-velocity zone may arise in places in the upper or middle crust because of variations in composition, but it would be unlikely to exist over an area of even continental extent

In search of a meaning

from a Correspondent

THE first plenary meeting of the International Society for Research on Aggression, whose objects are as much educational as scientific, was held in Toronto on August 17 and 18 The constitution of the society refers to "the destructive and constructive aspects of aggression", but does not define aggression and in fact definition was the most prominent theme of the meeting, which was attended by fifty or so researchers (about one-fifth of the total membership) from fields as various as anthropology, psychiatry, and experimental psychology

On the question of definition, K Lagerspetz (Swedish University, Turku) pointed out that although most definitions include the delivery of noxious stimulation to another organism, this does not always apply in psychiatry or the social sciences She asked whether, nonetheless, unifying concepts exist for all the fields of behaviour to which the term 'aggressive' is applied In her view the notion of an 'aggressive drive' is not useful, and is critical of the frustration hypothesis and analyses in terms of learning and inhibitions

Adopting an historical perspective, J P Scott (Bowling Green State University, Ohio) suggested 'disaggregation', or disruption of normal social patterns, as a general concomitant of anti-social violence in both human and animal societies

The pluralism advocated by Lagerspetz was echoed in a discussion on classification and definition K E Moyer (Carnegie-Mellon University, Pittsburgh), S Rosenzweig (Washington University, St Louis) and P A Corning (Stanford University) all emphasised the heterogeneity of the phenomena with which members of the society are concerned The complexities of human behaviour in this regard were exemplified in a remark by J Paddock (University of the Americas, Oaxaca), during an absorbing communication on anti-violent and 'normal' communities in which he remarked that non-violent and anti-violent attitudes can be militant

Education as a possible unifying activity for the society was proposed, and briefly discussed Authentic information on anti-social violence in human communities and on the social and intolerant behaviour of animals, is much needed, notably by teachers in schools and universities, at present they have to contend with a flood of misinformation and entertaining but unscientific writing The refreshing rigor of the papers and discussions at this meeting suggest that the society could do much to remedy this situation

Lamb shifts from intensity measurements

from B P Kibble

PHYSICISTS at the University of Windsor, Canada have found an interesting new way to measure the small energy change known as a Lamb shift which is the effect of radiation reacting on the atom emitting it In their report Wijngaarden, Drake and Farago (*Phys Rev Lett*, **33**, 4, 1974), following the theoretical treatment of Drake and Grimley (*Phys Rev*, **A8**, 157, 1973), describe an indirect measurement in terms of an intensity ratio of the Lamb shift in the lowest excited states of hydrogen

Two of the states concerned, denoted as $2P_{1/2}$ and $2P_{3/2}$, have angular momentum associated with them which differs from that associated with the ground state An atom in either of these states can revert to the ground state by emitting photons of slightly different energies as, because of relativistic corrections, these states have energies differing by a small amount ΔE A third state denoted as $2S_{1/2}$ also differs in shift $\mathscr{S}$ approximately equal to 0 1 energy from the $2P_{1/2}$ state by a Lamb ΔE, but has associated angular momentum which is the same as that of the ground state so that an atom in it does not radiate a photon In previous measurements transitions between this S state and the P states resulting in photon emission could be induced by a radiofrequency field whose frequency matches, and is a measure of, the energy differences $\mathscr{S}$ and $\Delta E-\mathscr{S}$

The alternative approach of the Canadian workers is to note that when the 'pure' states are mixed by the presence of an electric field, the total photon emission is no longer equal in all directions, but is greater in the direction of the electric field than in the perpendicular direction The ratio is related to $\mathscr{S}/\Delta E$ and since ΔE is already known very precisely from radiofrequency spectroscopy, $\mathscr{S}$ can be deduced

Measuring radiative intensities, even when only the ratio is required, does not seem likely to be very accurate but there are particularly favourable features in this case The photons are energetic enough for a high proportion of them to eject countable single electrons from a detector surface and, when increased by an electron multiplier, the resulting pulses of electrons can be accurately counted If the difficulties of variation of this proportion and of the discriminator level which differentiates between large signal pulses and small noise pulses can be overcome, the direction of the electric field can be controlled sufficiently precisely, the results can be extrapolated to a condition of zero electric field strength and corrected for the occasional coincidence of pulses, then an accuracy comparable to that obtained by radiofrequency methods may be attained So far Wijngaarden and his colleagues report an accuracy of about 1%, which is two orders of magnitude less than that obtained by radiofrequency methods, but the prospects for improvement are encouraging The method may well be suitable for Lamb shifts in heavier hydrogen-like ions where a method based on the total photon emission from the S state by application of an electric field has hitherto been used

Early mammalian development

from Michael Balls and Arthur Wild

RECENT dramatic technical developments have enabled the mammalian embryo, which normally remains hidden within the female reproductive tract, to be viewed and manipulated These advances were reflected in the programme of the twenty-seventh meeting of the British Society for Developmental Biology, on the early development of mammals, held at the University of East Anglia on September 9–12 In a group of papers on *in vitro* techniques, Dr D Whittingham (University of Cambridge) dealt with problems related to the fertilisation, culture and storage of mammalian ova, and Dr M Kaufman (Weizman Institute, Revohot) reviewed recent developments in experimental parthenogenesis and considered possible reasons for the death of parthenogenetic mouse embryos in early post-implantation stages Dr A McLaren (University of Edinburgh) discussed the culture of embryos over the period when implantation would normally be occurring *in vivo*, and the culture of post-implantation rat embryos was described by Dr C Steele (University of Cambridge)

Contrary to Dalcq's rigid predetermination idea, experiments reviewed and described in papers by Dr I Wilson (University College of North Wales, Bangor) and Dr S Kelly (University of Oxford) clearly demonstrated the totipotency of the blastomeres up to and including the eight-cell stage, pointing to a labile, epigenetic control of the differentiation of two distinct cell populations (trophoblast and inner cell mass) The further development of these two cell types was considered by Dr R Gardner (University of Oxford), using chimaeras, and by Dr J Ansell (University of Cambridge) and Dr M Sherman (Roche Institute, Nutley, New Jersey), using *in vitro* trophoblast outgrowths

Abnormalities of development can often be used to throw light on mechanisms operating in normal development, mutations in the complex T locus of the mouse are being studied to this end Dr N Hillman (Temple University, Philadelphia) proposed that metabolic errors elicited by mutant *t* alleles are involved in embryonic lethality, whereas Professor D Bennett (Cornell University, New York) suggested that the T locus controls a specific cell surface antigen which is of great significance in development

The expression of paternal antigens, especially those of the H-2 locus, could have important immunological consequences in development One theory as to why the mother does not normally reject the foetus as an allograft is that paternal antigens are not expressed on trophoblast cells, and Dr D Billington (University of Bristol) discussed the evidence for this hypothesis Antigen expression was also the subject of a paper by Professor F Jacob (Institut Pasteur, Paris), but he described experiments on teratocarcinoma cells, which also have great potential for studies on the basis of normal developmental mechanisms Dr D. Solter (Wistar Institute, Philadelphia) described methods for producing benign teratomas and re-transplantable teratocarcinomas, and the possible immunological factors regulating them Dr M Evans (University College, London) dealt with the characteristics of established cell lines derived from teratocarcinomas

Much is now known about the synthesis of various macromolecules and the fine structural changes that occur in the pre-implantation embryo The genetic activity underlying these events as expressed in the nuclear and mitochondrial genome was considered by Dr L Pikó (Pasadena, California) Dr C Ford (University of Oxford) discussed the effects of genetic imbalance, and a model system for studying its consequences in certain strains of mice, Dr B Cattanach (Harwell) described sex reversal in mammals and the use of *Sxr* mice in attempts to understand this intriguing problem

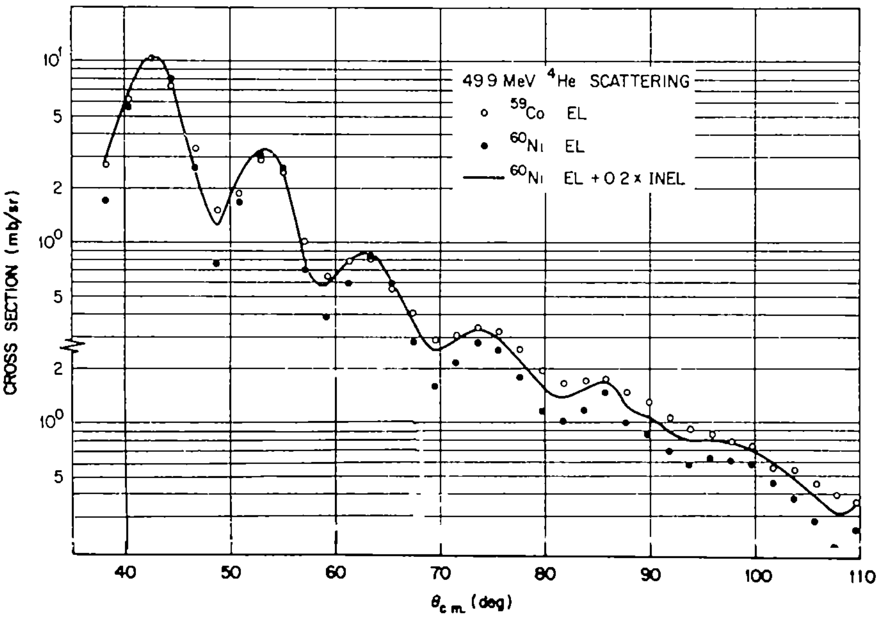
The next group of papers concerned the significance of cell interactions in the morphogenesis of organs, such as the kidney (Dr L Saxén, University of Helsinki), in pre-natal erythropoiesis (Dr R Cole, University of Sussex), and in pre-natal lymphopoiesis (Dr M Ritter, University of Oxford) Dr E Pantelouris (University of Strathclyde) put forward the hypothesis that the thymus, as evidenced by the effects of genetic athymia, might have an endocrine function in female reproductive physiology, and, finally, Dr J McKenzie (University of Aberdeen) presented evidence that early cell death might be the underlying cause of human morphological abnormalities hitherto ascribed to so-called 'amniotic bands' The proceedings of the symposium will be published in the spring or early summer of 1975

Target-spin effects in elastic scattering

from P E Hodgson

MEASUREMENTS of the elastic scattering of helions and alpha particles by nuclei have shown that the differential cross sections for odd nuclei oscillate less strongly with angle than the corresponding cross sections for neighbouring even nuclei Since even nuclei have spin zero and odd nuclei have spins greater than zero it was at first suggested that this difference may be explained by a coupling between the relative orbital angular momentum **L** and the target spin **I** which can be represented by a **L.I** term in the optical potential This term is similar to the **L**σ spin-orbit term which is known to reduce the oscillations of differential cross sections But this explanation of the difference was rendered unlikely by Satchler's calculations (*Nucl Phys*, **45**, 197, 1963) of the magnitude of the **L.I** target-spin term, which showed that it is far too weak to account for the observed differences in the cross sections

It has recently been suggested by Satchler and Fulmer (*Phys Lett*, **50B**, 309, 1974) that another possible explanation may be found in the different shapes of neighbouring odd and even nuclei due to particle–core coupling According to this model the odd nucleus is considered as a single particle (or hole) coupled to states of the core with spin **L**, where the core is the adjacent even nucleus The coupling allows the valence particle to polarise the core and thus gives it a quadrupole moment, and the extra scattering from the quadrupole moment has the effect of smoothing the angular distribution of the scattering from odd nuclei



Differential cross sections for the elastic scattering of 49 9 MeV α particles by ^{59}Co and by ^{60}Ni showing the difference in the oscillatory structure The full line is the cross section for ^{59}Co calculated as described in the text from the cross section for ^{60}Ni plus auxiliary data, and agrees well with the ^{59}Co measurements

To test this explanation, Satchler and Fulmer measured the differential cross section for the elastic scattering of 49 9 MeV α particles by ^{59}Co ($I=7/2$) and by ^{60}Ni ($I=0$) As shown in the figure, the ^{60}Ni cross section oscillates significantly more than the ^{59}Co cross section

The theory of elastic scattering from odd and even nuclei taking into account the quadrupole moment scattering predicts that they are related by

$$\sigma_{\mathrm{EL}}(\mathrm{odd}) \approx \sigma_{\mathrm{EL}}(\mathrm{even}) + \sum_{L} K_L \sigma_{\mathrm{INEL}}\ (\mathrm{even},\ 0^+ \rightarrow L^+),$$

where K_l is a coefficient that can be found from the measured 2^L-pole moments of the ground state of the odd nucleus and the measured strength of the electromagnetic excitation of the corresponding state in the even nucleus The inelastic cross section σ_{INEL}(even, $0^+ \rightarrow L^+$) for the excitation of an L^+ state in the even nucleus is also known experimentally It is thus possible to calculate the elastic scattering from an odd nucleus from measurable properties of the neighbouring even nucleus in a direct way using a simple model

The ^{59}Co cross section was found in this way from the ^{60}Ni cross section and the appropriate inelastic scattering and electromagnetic transition data, and the results are shown by the full line in the figure This agrees very well with the measured results for ^{59}Co, indicating that quadrupole moment scattering is indeed responsible for at least the major part of the difference between the scattering from ^{59}Co and ^{60}Ni

Further calculations showed that the same model is also able to account for the observed differences between the elastic scattering of 60 MeV helions by ^{59}Co and ^{60}Ni It is likely that it could account for other odd–even differences that have been observed in elastic scattering, except for nuclei with $I=\frac{1}{2}$, for which quadrupole moment scattering is zero Examples of this occur for ^{89}Y and ^{207}Pb, and such nuclei are therefore suitable for the study of target-spin effects

This work sheds further light on the character of the optical potential It would presumably be possible to fit the observed difference between the elastic scattering from odd and even nuclei by including an **L.I** term in the potential and arbitrarily adjusting its strength This strength would, however, vary from nucleus to nucleus depending on the deformation and would be much greater than the value calculated from the microscopic theory of the optical potential This is clearly unsatisfactory, and a much better way of analysing the data is provided by the present work, which shows that a more detailed account of the physics of the interaction enables the data to be understood in a systematic way without arbitrary parameters

Ins and outs of cell membranes

from a Correspondent

THE chemical assymmetry of biological membranes in the distribution of lipids, proteins and carbohydrates at each face of the lipid bilayer was no doubt implicit in the early membrane models Recently this proposal has been made explicit, largely through the introduction of a variety of reagents that react to form stable derivatives of membrane components without penetrating the lipid bilayer The usual experiment is to react in this way intact cells, in which case only components accessible at the outer surface are labelled, or lysed cells when additional sites located at the cytoplasmic side of the surface membrane also become available for reaction

In a recent issue of the *Journal of Molecular Biology* (**87**, 541–562, 1974) Whiteley and Berg describe two new homologous imidoesters that react under physiological conditions in respectively a non-penetrating or a completely penetrating manner These reagents can be tagged with ^{3}H or ^{14}C so it is possible to doubly label the membrane in such a way that reactive sites situated at the inner or outer face or both faces of the membrane can be identified Components that span the membrane can be distinguished from those having copies occupying sites on both sides of the membrane by the sensitivity of the former to proteolysis of intact cells which reduces the size of components accessible on the outer surface

The non-penetrating reagent described by Whiteley and Berg, (IAI) isethionyl acetimidate ($CH_3CNH\ OCH_2CH_2SO_3^-$), is polar and lipid insoluble, the penetrating reagent (EAI), ethyl acetimidate ($CH_3CNH\ OCH_2CH_3$) is relatively non-polar and lipid soluble The product of the reaction of these reagents with amino groups is, however, the same amidine Reaction of intact human erythrocytes or lysed erythrocyte ghosts with EAI labels haemoglobin to the same extent, whereas the haemoglobin of intact cells is only 0 6% as reactive to IAI as free haemoglobin Therefore, EAI penetrates the membrane and reacts with cytoplasmic proteins at about the same rate as occurs extracellularly in buffer at *p*H 8 The membrane amino groups reacting with these reagents are in proteins or lipids

When intact cells are labelled with IAI only a fraction (about 2×10^6 sites per cell) of the amino groups substituted by reaction of the reagent with lysed cells are labelled and 90% of the counts are in protein There are nearly 20 times as many reactive protein amino groups and nearly 100 times as many lipid amino groups (phosphatidyl serine and phosphatidyl ethanolamine) on the inner membrane surface as on the outer surface of the erythrocyte These findings are in general agreement with assignments made by other labelling methods

The use of these new imidoesters however makes an important contribution to recent discussion of the validity of surface labelling techniques Much of the earlier work has used low concentrations of reagents, and since the labelling often does not proceed to saturation, sites on the inner membrane surface of intact cells may be weakly labelled even by totally penetrating reagents because the large excess of cytoplasmic protein, for example haemoglobin, mops up any reagent appearing at the cytoplasmic face of the surface membrane Therefore, it is not always completely clear whether the non-reactivity of cytoplasmic face membrane components to a particular reagent means that the reagent is incapable of penetrating the lipid bilayer This argument has been considered to explain the preferential labelling of outer face components by low concentrations of reagents that were supposed to be completely penetrating Whiteley and Berg show, however, that the labelling of free membrane amino groups by EAI is the same in intact cells or lysed cells Labelling of spectrin polypeptides, for example, proceeds to the same extent by exposure of intact cells to EAI or lysed cells (but not intact cells) to IAI ($3\ 0\times10^7$ and $1\ 2\times10^7$ amidines per cell respectively) Clearly then, these proteins (which include spectrin and a number of smaller proteins) exist exclusively on the inner surface face and are fully reactive to a completely penetrating reagent added at saturating concentrations Whiteley and Berg also show convincingly that membrane reorganisation caused by lysis must be slight since intact cells treated with EAI do not incorporate more EAI into membrane components on further reaction with fresh reagent added to lysed cells It does not, of course, prove that limited reorganisation might not occur during hypotonic lysis, for example, to make certain surface groups more reactive with the non-penetrating imidoester IAI This can only be decided by mapping the positions of amidine residues along membrane polypeptides labelled under the different conditions

As an alternative to small chemical reagents, surface labelling with enzymes (lactoperoxidase or galactose oxidase) is extensively used The reaction of membrane components with galactose oxidase is usually followed by labelling of the aldehyde group generated at C6 of galactose residues by the enzyme with ^{3}H-NaBH$_4$ In a new development with wide practical potential, particularly for the electron microscopic visualisation of membrane glycoproteins, Heitzmann and Richards (*Proc natn Acad Sci, U S A*, in the press) react the aldehyde groups with biotin hydrazide to form hydrozones The bound biotin is in turn located by ferritin-linked avidin The association constant of the biotin–avidin interaction is about 10^{15} M^{-1}, that is several orders of magnitude larger than the interaction with a ferritin–lectin, another favourite reagent for localisation of membrane carbohydrate groups Staining of membranes containing fixed biotin is therefore extremely rapid, minimising non-specific background If required the galactose oxidase treatment can be dispensed with and a suitable derivative biotinyl-N-hydroxysuccinimide ester is reacted directly with protein amino groups

The range of enzyme reagents has been extended by the introduction of enzymatic phosphorylation of proteins for the labelling of surface proteins (Kinzel and Mueller, *Biochim biophys Acta*, **322**, 337–351, 1973) Protein kinases are ubiquitous enzymes transferring the terminal phosphate of γ-^{32}P-ATP to serine or threonine residues of protein, and usually show a fairly broad substrate specificity Kinzel and Mueller find that phosphorylation of intact HeLa cells by exogeneously added protein kinase from rat skeletal muscle labels about 15 polypeptide subunits separable by SDS-polyacrylamide gel electrophoresis A heavily labelled component has an apparent molecular weight of about 55,000 One possible complication using this procedure is the presence of endogenous protein kinases in some cell membranes The intrinsic phosphorylation of proteins by these kinases probably accounts for the slow appearances of hydroxylamine stable phosphates when cells or isolated plasma membranes are incubated alone with γ-^{32}P-ATP Phosphoproteins containing aryl phosphate bonds formed during the process of ATP hydrolysis catalysed by surface located ATPases are likely to be less of a problem, particularly if the reaction conditions are arranged so that any inorganic phosphate released from γ-^{32}P-ATP by a surface enzyme is trapped in an external medium containing a high concentration of cold phosphate

articles

Indian astronomy in the era of Copernicus

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An observatory, in use in India during the time of Copernicus, was part of an astronomical tradition dating back to the second millenium BC

In the wake of the quincentenary celebrations for the great Polish astronomer-philosopher Nicolaus Copernicus, it seems appropriate to recall that during his lifetime Indian astronomical methods were being used in Newminster, England as well as in other parts of Europe[1] At about that time there was also a functional observatory, called *Man Mandir*, in Benares, India, the fame of which attracted European scholars until the nineteenth century

Scholars and traders went to India from Europe in search of knowledge as well as merchandise much earlier than the sixteenth century, and more steadily after the voyage of Vasco da Gama to Calicut in 1498 During the eighteenth century wealthy patrons and learned societies, such as the Royal Society of London and the Royal Society of Paris, sent scholars to bring back accounts of what they saw

Among the British scholars visiting the Benares observatory was the distinguished Sir Robert Barker (1729-89) a member of parliament, who gave an eye-witness account

> "I saw a number of instruments yet remaining, in the greatest preservation, stupendously large, immoveable from the spot, and built of stone, some of them being upwards of twenty feet in height and although they are said to have been erected two hundred years ago, the graduations and divisions on the several arcs appeared as well cut and as accurately divided, as if they had been the performance of a modern artist The execution in the construction of these instruments exhibited a mathematical exactness in the fixing, bearing and fitting of the several parts in the necessary and sufficient supports to the very large stones that composed them, and in the joining and fastening each into the other means of lead and iron "[2]

Dr Reuben Burrow (1749-92), assistant to Professor Maskelyne the Astronomer Royal at Greenwich, was given the respectable position of Chief Engineer of the East India Company in 1782 In an article entitled, "Hints concerning the observatory of Benares", which is preserved in the British Museum Papers of the Right Honourable Warren Hastings, the first Governor-General of Bengal, from 1774-85 (reprinted in ref 3) Burrow observed

> "In all the Grecian, Roman and Egyptian remains there does not appear to be the least vestige of an observatory but fortunately for astronomy there is a large quadrant existing at Benares which from the intent of its construction must necessarily have been placed in the plane of the meridian when the observatory was erected, and as this quadrant is an immovable structure of solid massy stones, and consequently not liable to vary its azimuth, or bend like European quadrants, the transits and altitudes of a number of stars may be taken with it, by a proper contrivance, and its position with respect to the meridian and equator and c, found out with the greatest exactness, from whence many useful conclusions may be drawn "[3]

As late as 1823, the writers of the *Encyclopaedia Britannica* considered *Man Mandir* to be one of the five most celebrated observatories of the world, the other four being the Greenwich observatory built by order of Charles II in 1676, the Paris observatory built by order of Louis XIV in 1667, Tycho Brahe's observatory in the Island of Hven, construction of which began in 1576, and the Peking observatory rebuilt by order of the Ch'ien Lung, the Emperor of China in 1744 (ref 4)

First-hand information available to the Royal Society of London from scholars and experts sent to India led the *Encylopaedia Britannica* to state

> "The science for which the bramins, however, were most remarkable, is that of astronomy, and in this their progress was so great, as even yet to furnish matter of admiration to the moderns "[5]

The interest in astronomical sciences in India, which goes back to the Vedic period, about 1500 BC, was probably cultivated and developed in connection with the religious need for consecrating ceremonies and sacrifices at specific times and seasons The Vedic rites of Agni-cayana (fire altars) required the construction of shrines of strictly accurate geometrical dimensions where sacred ceremonies were to be performed at a specific and exact time and date[6]

Since the determination of the units of time is contingent upon the accuracy with which the celestial phenomenon can be observed, the needs to study problems of mathematics and astronomy arose simultaneously This resulted in the determination of solstices and equinoxes, division of the Sun's yearly path into 360 divisions and of the zodiac into twelve equal parts, the discovery of the planets and so on The pole star was located as the pivot around which the celestial sphere rotated The Sun was considered the central point, the director of the Earth, around which all the planets revolved "May the resplendent sun that comes from the centre of the expanse of water of the vast ocean, purify me ", and "The sun generates all the earthly directions one by one and controls the seasons Only the sun is the Lord of our universe "[7]

The growth of astronomical sciences continued with the compilation of the first systematised text of astronomy, *Vedanga Jyotisha*, by Lagadha in about 600 BC (dates vary) This text deals with planetary motions, describes eclipses and divides the ecliptic into 27 or 28 equal parts, called nakshatras The nakshatras determined the Moon's position in relation to stars each day, the Moon completing its sky journey in 27 or 28 days[8,9]

The acquisition of some of this knowledge during the Vedic period might have been a result of interaction with the Mesopotamian culture, just as there is evidence of the interaction of Greek and Indian Sciences since the early Christian era It is well known that Greek scholars visited India from time to time, and some of them settled there, contributing towards the development of what may be termed the Indo-Greek Sciences[10] The concept of Earth being the centre of the Universe was the contribution of the Greeks to the Indian thought

The *Paulisa Siddhanta*, one of the five treatises on astronomy compiled in Sanskrit by Varaha Mihira in the fifth century AD uses Greek methods of computation, for example, in the *Table of Sines*, the subdivision of the radius is into 120 instead of 60 parts as prevalent in India This work also mentions the longitude of Alexandria on one hand and of Ujjain and Benares on the other, thus attesting to the significance of Benares (as well as Ujjain) as the centres of astronomical sciences in ancient India[9]

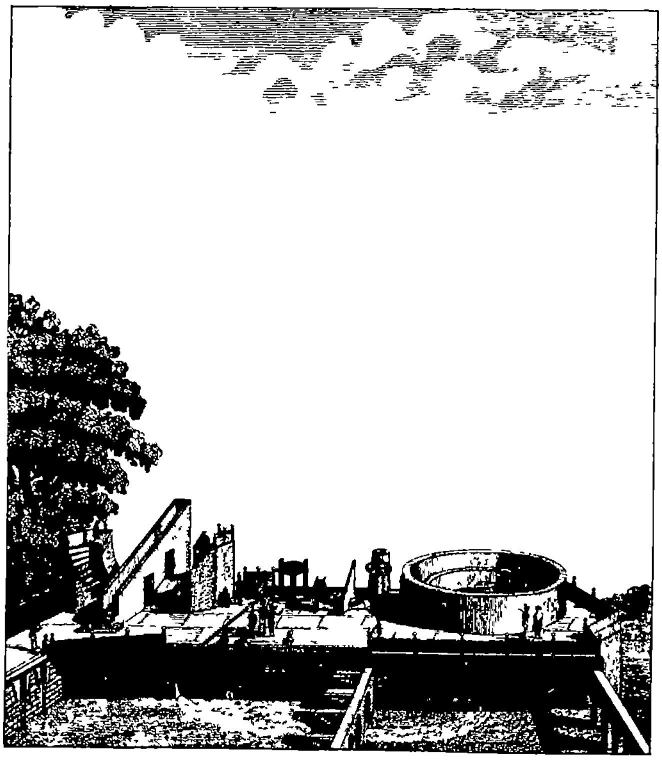

Fig 1 The observatory at Benares, from the *Encyclopaedia Britannica* of 1778 The drawing is by Sir Robert Campbell, chief engineer of the East India Company

By the seventh century, the Indian astronomers had discussed astronomical constants and sine tables, explained motions of the Sun, the Moon, and the planets, charted eclipses, and explained the theory of rotation of the Earth Alberuni, the Persian scholar, who visited India in the eleventh century AD paid a tribute to a seventh century Indian astronomer "Brahmagupta holds a remarkable place in the history of Eastern civilisation It was he who taught the Arabs astronomy before they became acquainted with Ptolemy, for the famous Sindhind of Arabian literature frequently mentioned but not yet brought to light, is a translation of his Brahmasiddhanta "[11] Indian astronomy was introduced in the Middle East after the time of Caliph Al-Mansur (eighth century AD) and subsequently reached Europe Until the twelfth century AD, the two well known centres of astronomical research were Ujjain and Benares Ujjain had the same reputation in ancient India as Greenwich has in the modern world There is, however, no record of astronomical instruments at Ujjain earlier than those installed by Jai Singh in 1734, and even these have disappeared today

Ancient records were lost because the astronomical sciences, along with the others, were dealt a severe blow after the invasions of Shihabuddin Muhammad Ghouri and his General Qutubuddin Aibak in the latter part of the twelfth century AD In 1194 Benares was ransacked and almost every monument of ancient Indian civilisation was irretrievably damaged The annihilation was so complete that no records of ancient learning were spared from destruction In 1199 Vihara (Bihar), a centre of learning and famous as the site of a college, was laid in ruins The great university of Nalanda was destroyed, its spacious library burnt to ashes, and practically all Buddhist literature and monks exterminated The religious fanaticism that continued in the thirteenth and the fourteenth centuries resulted in the collapse of astronomical activity during this period, the last celebrated Indian astronomer being Bhaskara II, 1150 AD This historical background is essential to an evaluation of the contradictory information now available about the Benares observatory

Astronomical activity was revived at the beginning of the Copernican era Works of mathematical astronomy and astronomical calculations, though limited to computation of data and tables and commentary of previous works, were compiled by Gangadhara (1420), Ganakananda (1447) and Makaranda (1478), followed by further works in the sixteenth century by Jnanaraja (1503), Ganesa (1507) and Vallala (1510)[12] European traders and scholars visiting India in the sixteenth and seventeenth centuries witnessed this last flicker of astronomical work It was this information that attracted eminent European scholars in the eighteenth century

The axe of fanaticism again fell in 1669 when the last of the Great Moguls, Aurangzeb Alamgir, sacked Benares "to demolish all the schools and temples of the infidels and to put down their religious teaching and practice"

The origins of Benares observatory therefore are shrouded in obscurity It is known that Vallala moved to Benares for astronomical studies in about 1500 AD, which might account for the existence of an observatory there Sir Robert Barker was told in 1772 that the observatory was constructed on the orders of Emperor Akbar in the late sixteenth century A Campbell and T D Pearse, who visited the observatory in the 1770s stated that the observatory was built two centuries previously by Raja Man Singh son of Jai Singh, and named *Man Mandir* after him J L Williams and William Hunter, who visited India in 1793 and 1798 respectively, on the basis of a newly discovered document, *zeej Muhammadshahi*, the authenticity of which is questionable, considered that the observatory was built in 1743 "for the repose of holy men and pilgrims never used, nor capable of being used for any nice observations (that it was) built more for ostentation than the promotion of useful knowledge" G R Kaye (whose observations are considered incorrect by some authorities) in "A guide to the old observatories", published in 1920, concurred with the opinions of Williams and Hunter, surmising that the Benares observatory was one of the five Jantar Mantars (observatory) erected by Raja Jai Singh of Amber in 1737, the four others being built at Delhi, Ajmer, Ujjain, and Mathura[12] Prinsep and Tavernier who visited India in the seventeenth century, during the lifetime of another Raja, Jai Singh, considered that he had built the observatory[4]

All the above sources—those who believe that the observatory was built in the sixteenth or the seventeenth century or those who believe that the observatory was built in the eighteenth century—nevertheless agree that it was constructed on the roof of an old building It is hard to conjecture why anybody would wish to construct new observatory on the roof of an old building, or why that particular building was chosen for the placement of astronomical instruments It is obvious that Emperor Akbar, Raja Man Singh or Raja Jai Singh (whoever he might have been, as there were several of them), were interested in restoring a historical observatory rather than building a new one If they wanted to build a new observatory, and that particular building in Benares had no value, they would have done so, as they certainly did not lack the financial resources, closer to their capitals, rather than selecting an old damaged building, that too in Benares The above considerations including the fact that there has been a tradition of continued astronomical research in India going back to the ancient Vedic period leads to the conclusion that there was an observatory in Benares which was damaged during Shihabuddin's invasion of 1194 Whatever remained of the observatory was of interest to Indian astronomers who carried on limited observations there, and were seeking a patron to restore and renovate it When political normality returned in the sixteenth century, the old observatory was restored If the stories of Jai Chands are true, this again underlines the fact that the observatory was damaged and rebuilt several times, which attests to the historical significance of Benares as an ancient city of astronomical learning John Call, FRS, while looking at the signs of the zodiac on an ancient monument, observed "These buildings and temples were places of residence and worship of the original Bramins, and bear the mark of great antiquity, having perhaps been built before the Persian conquest," or, in the words of Barker, "it serves to strengthen the argument of supposing that the Bramins had a knowledge of astronomy before the introduction of Mahometanism into Hindostan (India)[2]

The meaning of *Man Mandir* is "Temple of the Mind," a more appropriate name for this ancient observatory This old observatory is purely of historic interest, as the methods of observation and the remaining instruments are of no value today Observations in the past were made with the naked eye using suitable devices for measuring the angles The telescope was unknown to Indian astronomers In spite of these limitations, some remarkable calculations were made in ancient India that led to the establishment of an astronomical epoch The Indian astronomers established an epoch when the Sun, the Moon and all the planets were last present in zero longitude that was in 3102 BC The period from one such epoch to the next, according to Aryabhata (fifth century AD) is 1,080,000 yr and is called a Yuga Four Yugas, the Krtayuga, Tretayuga, Dvaparayuga, and Kaliyuga make a Mahayuga, and 1,008 yugas make a kalpa The current quarter is the Kaliyuga, assumed to have begun at sunrise at lanka (a hypothetical place where the meridians of Ujjain intersect the equator) on Friday, February 18, 3102 BC The epoch of zero longitude is apparently useful in computing the mean longitude of a planet[13]

$$\text{mean longitude} = (R \times A)/C$$

where R is the number of revolutions of the planets in a yuga, C is the number of days in a yuga, and A is ahargana, that is the number of days elapsed since the epoch It is interesting to recall that the Newminster manuscript stated that the era of the flood was 3102 BC, the beginning of the kaliyuga era[14]

It was this type of information that impressed Professor John Playfair (1748-1819) to observe "Undoubtedly the ancient astronomers must have possessed the best instruments probably differing from modern ones, but fully as powerful "[4] The writers of the *Encyclopaedia Britannica* commented that the establishment of an astronomical epoch by the Hindoos (same as Hindus, Bramins, Indians) and the observation of this rare phenomenon—the conjunction of Sun, Moon and all the planets according to their mean positions—was a remarkable finding that had received respectable sanction by the French Academy of Sciences[6]

It is also curious that the date of Copernicus' birth, February 19, coincides with the date of the establishment of the astronomical epoch by the Hindus (Bramins), February 18, 3102 BC

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Rotation of the Italian Peninsula

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Palaeomagnetic measurements of Upper Cretaceous–Palaeogene limestones from the northern Apennines indicate that the entire crust of the Italian Peninsula has rotated anticlockwise through 43° This is the first direct evidence from Italy of rotation, which is of comparable magnitude to rotations observed elsewhere in western Europe

ANTICLOCKWISE rotations of the Iberian peninsula[1], Sardinia[2], Corsica[3], and the western[4] and southern[5-7] Alps have been inferred from palaeomagnetic studies of Permian to mid Tertiary rocks and from geological evidence[8] An anticlockwise rotation of the Italian peninsula has also been suggested The rotation of Italy fits some theoretical schemes of Mediterranean palaeogeography These include originally continuous, linear, E–W mountain belts[9] and microplates packed into the western apex of the Tethys embayment of Pangea[10] Evidence of rotation has been found in palaeocurrent directions in the Tertiary Apennine flysch[11], and from palaeomagnetic measurements in the southern Alps (Permian and Triassic)[5,6] and the southern Alpine foothills (Tertiary)[12]

Palaeomagnetic studies in the southern Alpine region cannot, however, be used as evidence for rotation of the Italian peninsula, because the two areas are separated by the Po Basin in which the base of the Pliocene is as much as 6,000 m below sea level[13] (twice the present depth of the western Mediterranean basins) These sediments have been strongly folded and faulted, indicating severe tectonic activity Nothing is known of the pre-Miocene tectonic history of the Po basin, thus it cannot be assumed that evidence from the southern Alps is applicable to the Italian peninsula

No previous palaeomagnetic studies have been carried out in the Italian peninsula A major difficulty is that the western part of the peninsula is predominantly covered by allochthonous sequences, and the autochthonous (?) eastern sequences contain no strongly magnetic rocks We here describe the results of palaeomagnetic studies on a weakly magnetic Upper Cretaceous–Palaeogene limestone formation in the autochthonous or

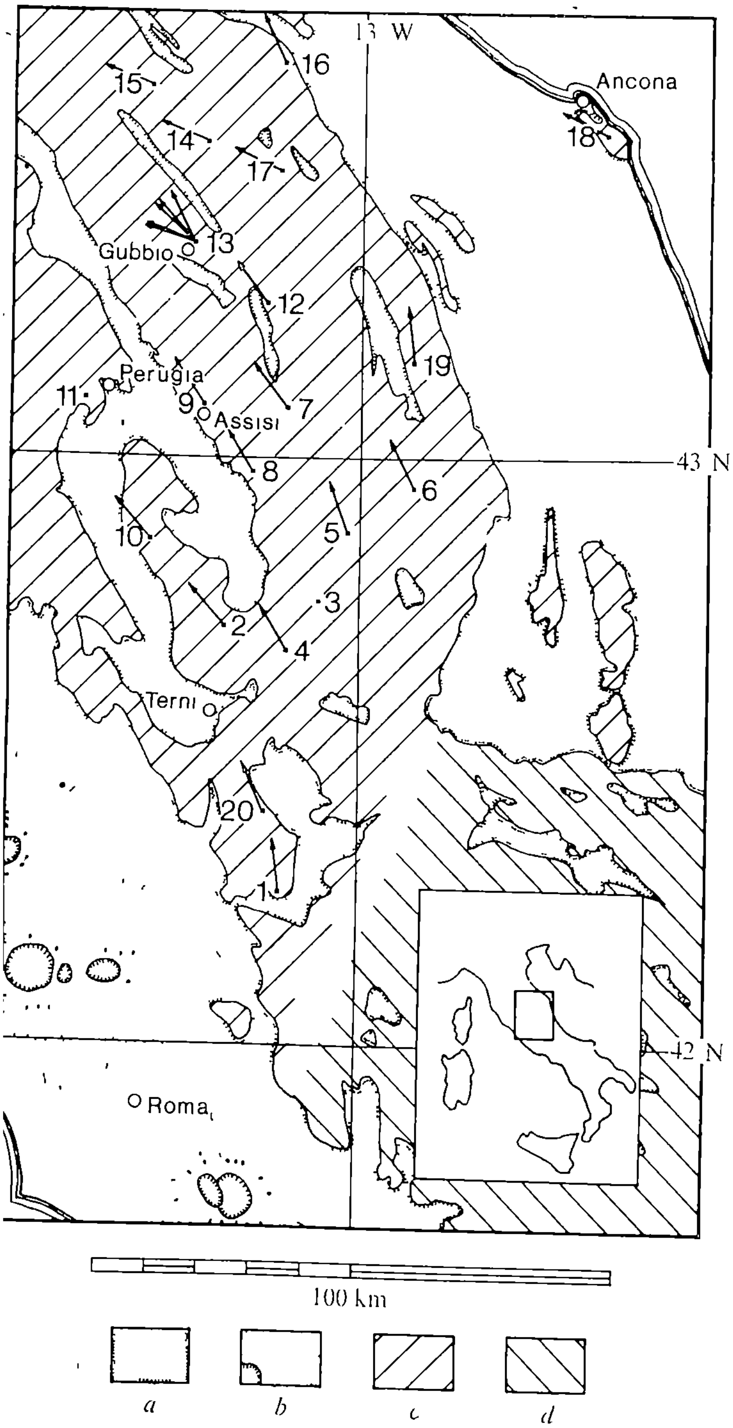

Fig 1 Location of sample sites, arrows show declination of remanent magnetisation *a*, Upper Tertiary–Quaternary sedimentary cover, *b*, Quaternary volcanic centres, *c*, Umbrian sequence (Mesozoic–Lower Tertiary), *d*, Abruzzi sequence (Mesozoic–Lower Tertiary)

para-autochthonous Umbrian sequence of the northern Apennines.

A neritic carbonate platform which occupied the area of the miogeosynclinal Umbrian sequence during the early Liassic subsequently broke up into a complex of seamounts and basins and underwent pelagic sedimentation during the remainder of the Jurassic and Cretaceous[14]. Increasingly terrigenous sedimentation in the Palaeogene led to the deposition of Miocene turbidite flysch, and to tectonic deformation in the late Miocene, the Pliocene and the Quaternary[15]. A long interval (Upper Cretaceous–Middle Eocene) is represented by the Scaglia Rossa, a pelagic limestone. Frequent outcrops and quarries expose this attractive stone. The time interval spanned by the formation, and its age and probably autochthonous character make it suitable for palaeomagnetic sampling.

Twenty different localities were sampled (Fig. 1). At locality 13 (the classic profile of the Bottaccione gorge at Gubbio[16,17]) the entire section is exposed, and we placed five sites here at different stratigraphic levels in order to measure the variation in palaeomagnetic directions during the depositional interval. Twenty-four sites were sampled. At each site, 7 to 14 oriented cores were drilled—a total of 180 cores, each of which gave two or three one-inch cylindrical specimens. The remanent magnetisations were very weak—10^{-6} to 10^{-7} gauss—but could be measured with very good repeatability. Susceptibilities were also very low—10^{-6} to 10^{-5} gauss oersted^{-1}.

A representative specimen was selected from each site and subjected to progressive alternating field (AF) demagnetisation in peak fields of up to 400 oersted in intensity. The demagnetisation characteristics were uniform for all the specimens (Fig. 2). After initial removal of an unstable component of magnetisation, the remanent intensity and direction were quite stable throughout the remainder of the demagnetising range, occasionally acquiring an anhysteretic component in 400 oersted. Computer analysis of the demagnetisation curves, utilising one index for directional stability and a separate index for overall magnetisation stability[18] enabled us to select the optimum demagnetisation field for each site. As the optimum field was always less than 200 oersted, all of the specimens were demagnetised in fields of 100 oersted and 200 oersted. Statistical analysis of the remanent directions for each site at each demagnetisation stage gave within-site circles of confidence with an average radius (α_{95}) of 10.7°.

The palaeomagnetic data were satisfactory at all but two of the sites. Local structural and stratigraphical complications obscured the significance of the data from site 3. At site 11, half of the sample directions were closely grouped near the mean normal directions of the other sites, but the other half had normal declinations and negative inclinations. It is possible that a partial field reversal was recorded in these samples. Alternatively, some of the samples may have been accidentally inverted. Because of these ambiguities the data from sites 3 and 11 were excluded from this preliminary analysis.

At site 13A a field reversal was apparently recorded. Most of the samples had normal directions, the remainder were exactly reversed in both inclination and declination. The normal equivalent directions were used in computing the site mean and other statistics.

Evidence from the five sites within locality 13 shows a progressive change in direction. The older sites, lower in the section, have more westerly declinations (by about 35°) than the younger sites. If supported by more detailed study, this implies that some rotation occurred during the mid Cretaceous–mid Eocene. Palaeontological dating of individual sites is at present available in only a few instances. These are in agreement with the established stratigraphy. Further palaeontological work is in progress and hopefully will enable us to refine the present picture.

The pink colour of the limestone at first suggested that secondary components of magnetisation resulting from haematisation may be important. Several tests indicated that this was not the case.

At site 9, samples were taken from adjacent pink and white sections of the same bed. Both sets of samples gave good quality data, similar in intensity, and with well grouped, reversed directions of magnetisation that did not differ significantly from each other. From this we conclude that the pink pigment does not provide an important contribution to the total remanence of the Scaglia Rossa. Thermal demagnetisation of pilot specimens from each site showed a notable decrease in the intensity and increased dispersion of the directions of weak remanence in the temperature range 550–600° C. These results suggest that the dominant magnetic mineral is probably magnetite, although the presence of a haematite phase cannot be completely discounted. Precise determination of the magnetic mineralogy is complicated by the difficulty of separating the magnetic minerals. Preliminary results indicate, however, that stable, secondary chemical remanent magnetisation is unlikely to be a serious factor affecting the remanent directions of the Scaglia Rossa.

The site mean directions of the remanence after alternating field demagnetisation in 200 oersted are shown in Fig. 3, the reversed directions were used in statistical analysis and in the calculation of the mean direction. The distribution of azimuths was uniform about the mean direction, and the distribution of angular differences from the mean direction was Fisherian at the 90% confidence level. The mean declination and inclination of the Scaglia Rossa were 320.1° and 45.4°, respectively, with $\alpha_{95}=5.9°$.

The mean locations of the late Cretaceous and early Tertiary poles for stable western Europe and the Russian platform are 83°N 194°E, and 75°N 161°E, respectively[19], the mean of these poles is at 79°N 178°E. The corresponding direction of site declinations are shown in Fig. 1. All but two of the sites were normally magnetised, the normal equivalents of the magnetisation at the centre of our sampling area would have a declination of 4.5° and an inclination of +52°.

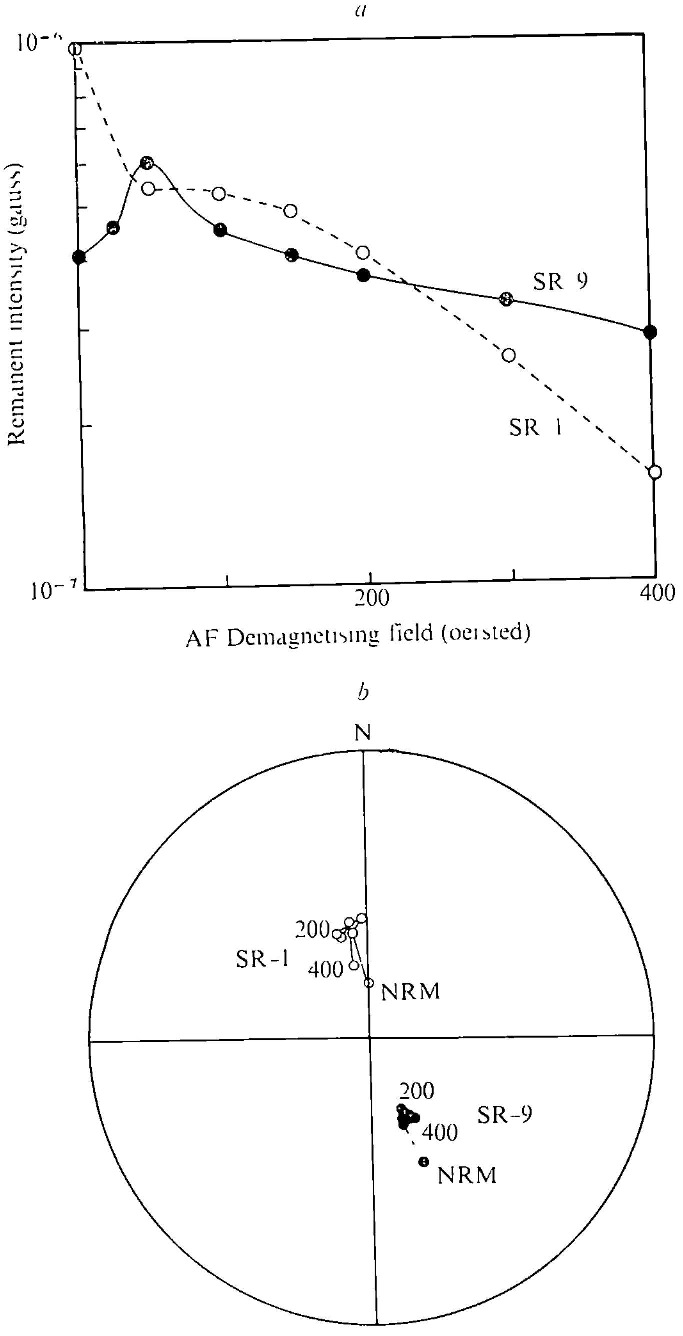

Fig. 2 Intensity (*a*) and direction (*b*) variations of normal (SR-1) and reversed (SR-9) specimens during alternating field (AF) demagnetisation. NRM, Natural remanent magnetisation.

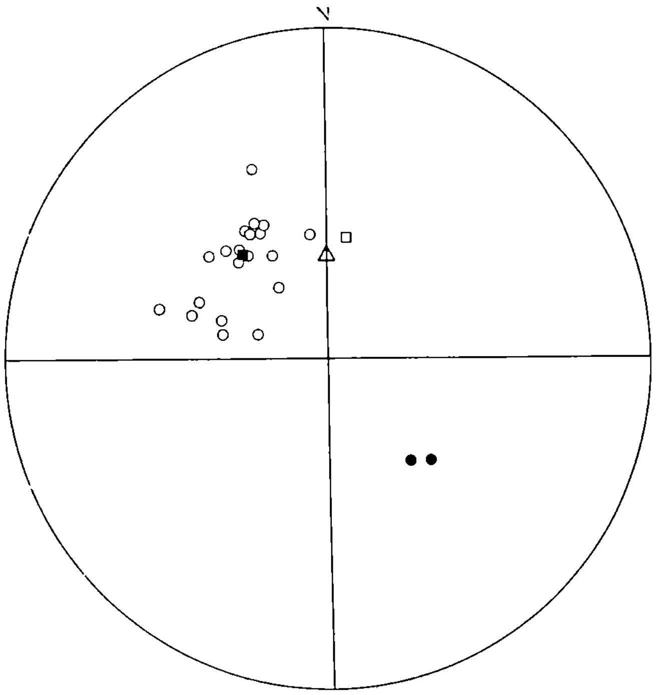

Fig. 3 Stereogram showing site mean directions of remanent magnetisation of the Scaglia Rossa after AF demagnetisation in 200 oersted. Open (solid) circles, normal (reversed) directions, open square and triangle, directions of the early Tertiary and Cretaceous (respectively) geomagnetic fields derived from the appropriate mean pole positions for stable Europe, solid square, mean direction for the Scaglia Rossa.

That direction differs by 6° in inclination and 43° in declination from the mean direction of our data. Thus, the inclinations are not significantly different and we cannot determine a palaeolatitude change with respect to stable Europe. There is, however, a significant declination difference, which is uniform at all but a few of the most northerly sites (Fig. 1).

The Scaglia Rossa carries a remanence which is probably not affected to any serious extent by secondary magnetisation. The relatively slow pelagic deposition, the long time interval represented by the formation, and the magnitude of the declination difference, show that this difference cannot be a result of secular variation. We therefore conclude that the rocks of the Scaglia Rossa have been physically rotated about a sub-vertical axis.

We envisage three ways in which such rotation could occur. First, there could have been rotation of the Mesozoic–Cainozoic sedimentary cover as a whole, relative to the underlying crust, if the cover is allochthonous above a major *decollement* surface. This, however, would require a relative displacement of about 100 km between the northern and southern parts of the studied area, nowhere have horizontal displacements of more than a few kilometres been observed. Second, the same rotational effect could be produced by simultaneous, independent, *en echelon* rotations of a series of small, allochthonous structures. Although we cannot dismiss this possibility, there is no evidence for it and this tectonic style has never been suggested for the Umbrian Apennines. Third, the entire crust of the Italian Peninsula may have rotated. This interpretation seems to be more compatible with the geological evidence than either of the others.

The 43° counterclockwise rotation is in the same sense as, and is of comparable magnitude to, rotations observed in other parts of the western Mediterranean. This is the first direct evidence that the Italian Peninsula fits this pattern.

A more detailed understanding of the tectonic significance of the rotation will emerge from additional palaeontological and palaeomagnetic sampling and measurement. It should be possible to determine whether deposition of the Scaglia Rossa occurred during the rotation, as suggested by the palaeomagnetic data from the five sites at locality 13 It should also be possible to test the hypothesis of oroclinal bending as the origin of curvature of the northern Apennines These studies are still in progress and a complete report will be presented later

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Bovine satellite I DNA consists of repetitive units 1,400 base pairs in length

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Bovine satellite I DNA was shown by its susceptibility to cleavage by restriction endonucleases to consist of direct tandem repeats 1,400 base pairs in length Renaturation kinetics indicate that this repeat is itself internally repetitious These data argue in favour of a DNA replication mechanism for the evolution of this satellite

THROUGH the light microscope, mitotic chromosomes look highly organised This organisation is revealed by means of the various cytochemical techniques that show characteristic banding patterns for each metaphase chromosome[1] Although the molecular basis of these bands is still unknown, it seems clear[2,4] that the local variations in staining intensity seen with a particular dye are a consequence of differences in packing density of the unit chromosomal fibre Regions selectively stained darker by Giemsa are shown to have a higher concentration of packed DNA than other chromosomal regions when measured by phase contrast microscopy[3,4] and by Feulgen staining[4,5] In many organisms, the regions adjacent to the centromeres of the chromosomes can be easily identified by these techniques Several authors have suggested that the dense packing of the chromosomal DNA in this area is due to the regularity of the DNA itself[4,6,7] The long order organisation of these units is therefore of interest

Wherever tested, the DNA of this centromeric heterochromatin[8,9] is largely composed of sequences of nucleotides repeated many times Because these sequences are simple and tandemly arranged, they can often be separated as 'satellites' from the bulk of the DNA by a variety of equilibrium centrifugation techniques Two approaches have been taken to study the organisation of satellite DNA First, using renaturation kinetics, Waring and Britten[10] found that mouse satellite renatured with a $C_0t\ \frac{1}{2}$ of 7×10^{-4} mol 1^{-1} s This renaturation data indicated a statistical complexity of 200-300 base pairs for the mouse satellite DNA, and similar data have been obtained for other satellite DNAs The second experimental approach to satellite organisation has been through various sequencing techniques The pyrimidine tract analysis of Southern[11] indicated that the guinea pig satellite α, contained a large amount of a repeating hexanucleotide Superficially, these results seem to be at odds with the observed kinetics of renaturation of satellite α DNA But Southern's data also showed that only 30% of the guinea pig α satellite was composed of the hexanucleotide and that the remaining sequences were considerably more complex It also seemed likely that the bulk of the DNA in this satellite was related to the basic unit by simple mutations and rearrangements Further studies indicate a similar situation in the mouse[12] and kangaroo rat[13] satellites The most complete sequencing data exist for the *Drosophila virilis* group satellites Gall and Atherton[14] have found that more than 93% of this satellite is of one heptamer A resolution between the two approaches discussed has not been reached, although it seems clear that the renaturation complexity cannot be taken as an actual repeat length

The data from all of these studies do not bear on the existence of a long range order to repeats within satellites Consider a tandem array where the repeat unit is (*baaaaaa* *b*) where both *b* and *a* represent a small sequence The renaturation rate of such a DNA would be dominated by *a* and would not indicate a higher order repeat Sequencing experiments without long overlapping fragments from this array might also leave this large repeat undetected This problem can potentially be resolved by use of restriction enzymes, which cleave double-stranded DNA at specific sequences If each repeat unit contains a single restriction sequence in it, the whole array of repeats will be cut to the length of one unit If the sequences of type *b* contain a restriction sequence, but those of type *a* do not, long fragments will be generated which reflect the separation between *b*s, that is the long order repeat If the site occurs more than once, fragments will be generated, the molecular weights of which will add up to the repeat length

Britten and Smith[15] have determined that 10% of bovine DNA consists of "highly repetitive" sequences, furthermore, this genome contains four distinct classes of satellite DNA[16,17] Kurnit *et al*[16] have found that the four satellites are located on the autosomal chromosomes and in the region of centromeric heterochromatin The major satellite[16–19], which has been mounted on an oriented platform. To obtain a long exposure on Her X-1 and also the background counting rate, the telescope was inclined at 17° to the zenith and programmed to point due north for 7 min and 8° east of north for the following 7 min; this cycle of orientation was continued throughout the flight. In-flight calibration of the detectors was carried out with a ^{241}Am source. Pulse heights in the energy range 21 to 125 keV were sorted into 15 energy channels and telemetered, together with aspect information. The time of occurrence of each event was recorded with an accuracy of 1 ms.

The procedure for evaluating the efficiencies as a function of time, and the reduction of spectral data, have already been described (ref. 1 and references therein). In the energy range 21.5 to 81.7 keV a total of 11,561 ± 1,122 excess counts attributable to Her X-1 were recorded in a total exposure of 5.7×10^5 cm^2 s. The spectral data and our earlier results are plotted in Fig. 1. The fluxes observed in the March experiment are about a factor of two lower than the fluxes observed in the December experiment. The December data can be fitted with either a power law with an exponent −2.7 or with a thermal spectrum characterised by a kT of 23 keV. The March results could be fitted with a power law of exponent −2.3 or a thermal spectrum with a kT of 30 keV. Combining the satellite observations below 20 keV and other balloon observations (Fig. 2) we find that a plasma temperature of 23 keV ($T = 2.7 \times 10^8$ K) fits reasonably well the entire data over an energy range 2 to 80 keV. The scatter in Fig. 2 arises because the observations were made at different phases of the binary and 35-d cycles. One significant feature is the steep fall in the intensity beyond 80 keV. Such a steepening was also reported by Ulmer *et al*[2,3]. The anomalously high value of Sharma *et al*[4] for intensities in the energy band 80 to 100 keV is not supported by our observations. If the intensity was really high it should have been seen with high statistical significance in our experiment. There is some evidence in the present experiment for variations in intensity in the 20 to 60 keV band by a factor of about two within the time scale of observation of ~70 m.

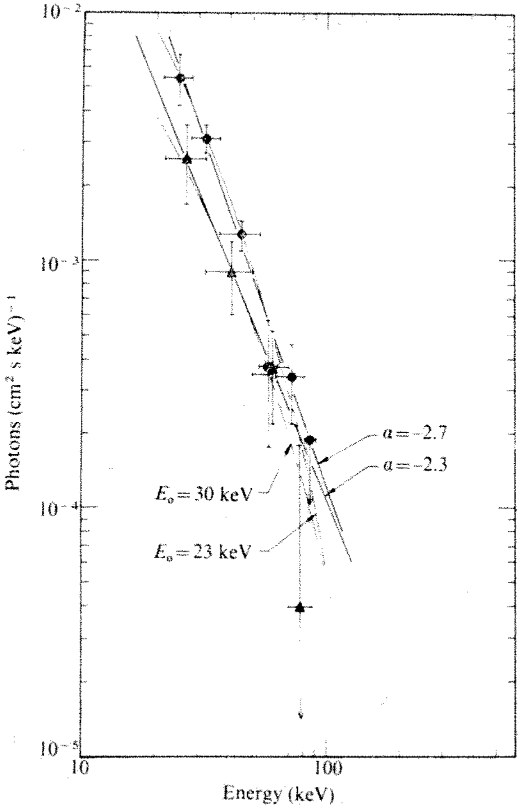

Fig. 1 Energy spectrum of Her X-1 in the hard X-ray band 20 to 90 keV measured in the experiments of March 9, 1973 and December 12, 1973. Best fits for power law (E^{α}) and plasma temperature (kT) for each of the two sets are also shown. ▲, March 9 (ref. 1); ●, December 12 (present work).

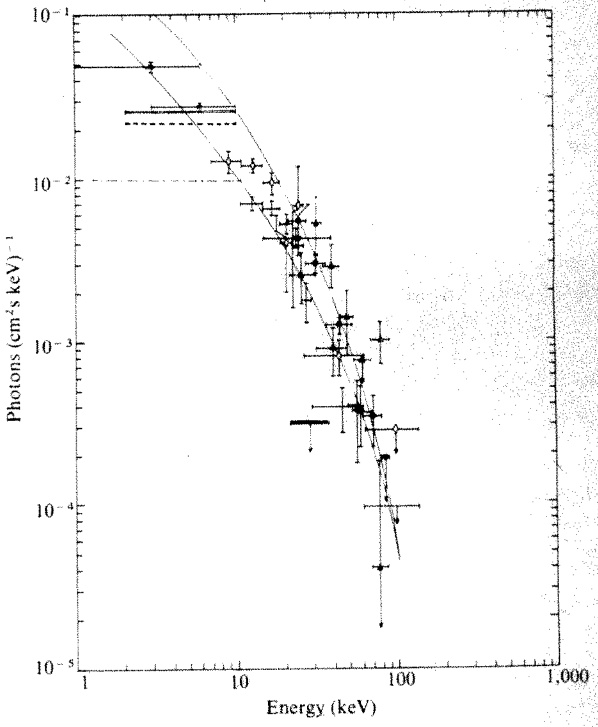

Fig. 2 Data on the energy spectrum of Her X-1 in the 1 to 100 keV interval, together with the fit for plasma temperature kT 23 to 30 keV as derived from our observations. Diamond and +, Refs 2, 3; —, Uhuru [5,6]; ▲, ref. 1; △, ref. 4; ×, ref. 9; ●, present work. Pulsation: – –, Uhuru; – · – · – ·, ref. 7; —↓, our work.

In our experiment the source 3U1706+32 did have a considerable exposure when observations on Her X-1 were made. But the intensity of the source in the 2 to 6 keV band is 1/25 that of Her X-1 (ref. 5) and even if the spectrum is flat, the contribution in the 20 to 45 keV will be small compared with that of Her X-1.

Since most of the contribution from Her X-1 was recorded in the energy channel 21.5 to 45.2 keV, the timing data in this interval were analysed to find the fraction of the pulsating component. The data were cast into 20 phase bins and none of the phase histograms with periodicity ranging from 1,235 to 1241 ms revealed a peak larger than 2.5σ. The χ^2 was as low as nine for 19 degrees of freedom (95% confidence). The data were also analysed in shorter time stretches of ~10 min each, and no significant peaks were seen in this analysis. These observations lead to an upper limit of 10% for the pulsating component if the width of the pulse is less than 400 ms. The limit is about the same even if the pulse width is as short as 60 ms. The data was also subjected to a Fourier analysis using an FFT algorithm; the power density distribution (Fig. 3) does not reveal any appreciable power in any of the frequencies investigated. In view of the negative results the algorithm was further tested by analysing self-generated data modulated with a known frequency.

The present upper limit of 10% for the pulsating component in the 20 to 45 keV band is to be compared with the lower limit of 80% in the 2 to 6 keV band[6] and 60% in the 2 to 11 keV band[7]. Ulmer *et al.*[2] have remarked that their preliminary analysis of the OSO-7 data failed to reveal any pulsations in the 7 to 60 keV band but further analysis may allow them to detect pulsations. Kurfess and Crosa[8] found no pulsed emission at energies greater than 100 keV, and could set a 2σ upper limit of 3.4×10^{-3} photons cm^{-2} s^{-1} in the 0.1 to 10 MeV range assuming a duty cycle of 35%.

So there is no evidence for a pulsating component at energies greater than 20 keV. As shown earlier the spectrum of the

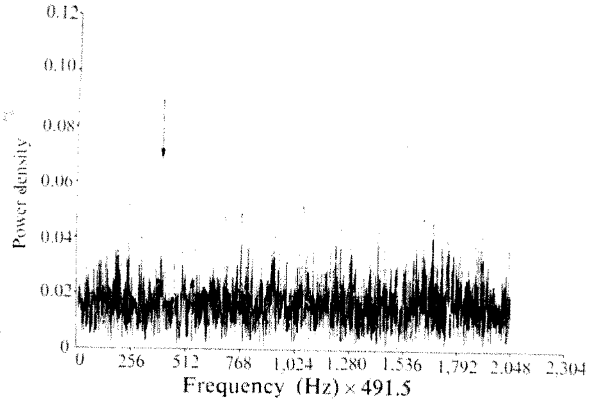

Fig. 3 Power density spectrum up to 2,048 Hz as obtained in the Fourier analysis of ~492 s of observation in the range 21.5 to 45.2 keV. The frequency corresponding to 1.24 s is shown by an arrow.

total X-ray emission over the entire energy range 2 to 80 keV can be fitted with a single plasma temperature of $kT = 23$ to 30 keV. The behaviour of the pulsating component as a function of energy is also shown in Fig. 2.

The possibility that the hard X rays arise from a different object close to Her X-1 can be ruled out from evidence[8,9] of the binary modulation and the 35-d cycle variation for the hard X-ray emission in phase with the modulation at lower energies.

The models which have been proposed to explain the features in the 2 to 10 keV range, regard Her X-1 as a rotating magnetised neutron star accreting matter from an eclipsing companion of larger size[10-14]. The influx of matter is constrained to a narrow region around the magnetic poles. The X-ray emission results from the transformation of the kinetic energy of the infalling matter into radiation which emerges preferentially in directions almost normal to the direction of the inflow. The observation of pulsed emission with the frequency of rotation is then a consequence of the direction of the magnetic axis being different from the rotation axis. The detailed pulse profile and in particular the pulse width, is determined by the angle between the two axes, the emission pattern and the direction of viewing. In such a model, if the hard X rays (20 to 80 keV) also originate from the same region as X rays of lower energy (1 to 10 keV) the pulsating fraction can be different only if the fan beam in hard X rays is considerably broader so as to reduce the extent of modulation during rotation. This, however, is contrary to the prediction of Davidson's accretion model[14] according to which the harder X rays come from a relatively narrow band at the base of the accretion funnel resulting in a narrower pulse width than in the X rays of 2 to 10 keV. Thus the present observations point to the need of a second component of X rays apart from the pulsating part.

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Direct observation of rock macerates

IN scanning electron microscopical (SEM) investigations of Precambrian sedimentary rocks, the risk of contamination during the preparation of rock macerates is extremely high. The technique described here and the use of stringent controls, have reduced to a minimum the degree of contamination by dust and microorganisms and, during preparation, enabled the recognition of artefacts from laboratory reagents.

In a preliminary study we used a dark, laminated, shaly sandstone from the Diabaig Formation of the Torridon Group, in north-western Scotland[1] (NG799605)[2]. This formation is believed to be older than $995 \pm 24 \times 10^6$ yr (ref. 3), and was selected because samples from other outcrops of this formation have already yielded microfossils[4-6].

Samples of rock were powdered with a hammer on a clean steel block and the powder was passed through a 600-μm sieve. Working in a sterile air flow, small samples of the powder were put into purpose-built wax stub tubes[7] in stoppered plastic test tubes, and treated with Schulzes' solution for 3 h to oxidise any exposed organic matter. Tests on spores of *Penicillium chrysogenum* showed that they were destroyed after only 30 min treatment; 3 h was, therefore, considered long enough to clean the

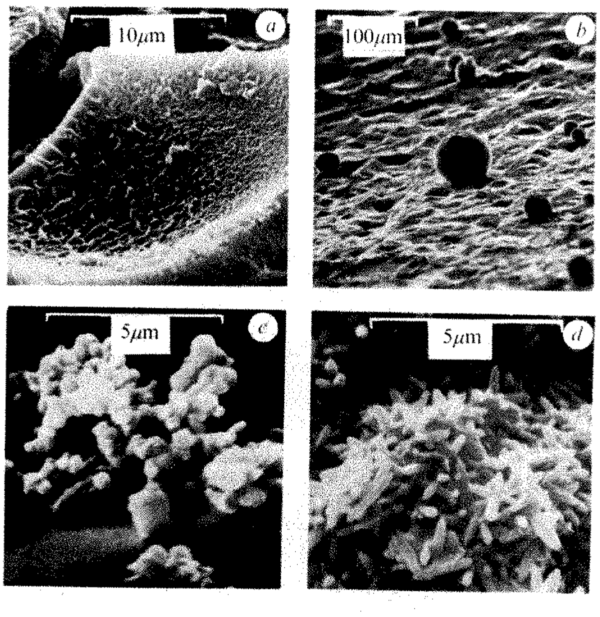

Fig. 1 *a*, Ornamented crust from the evaporation of laboratory distilled water; *b*, resin spheres from de-ionised water; *c*, residue after the reaction of hydrofluoric acid with silica; *d*, precipitated metal fluorides from a prepared rock macerate.

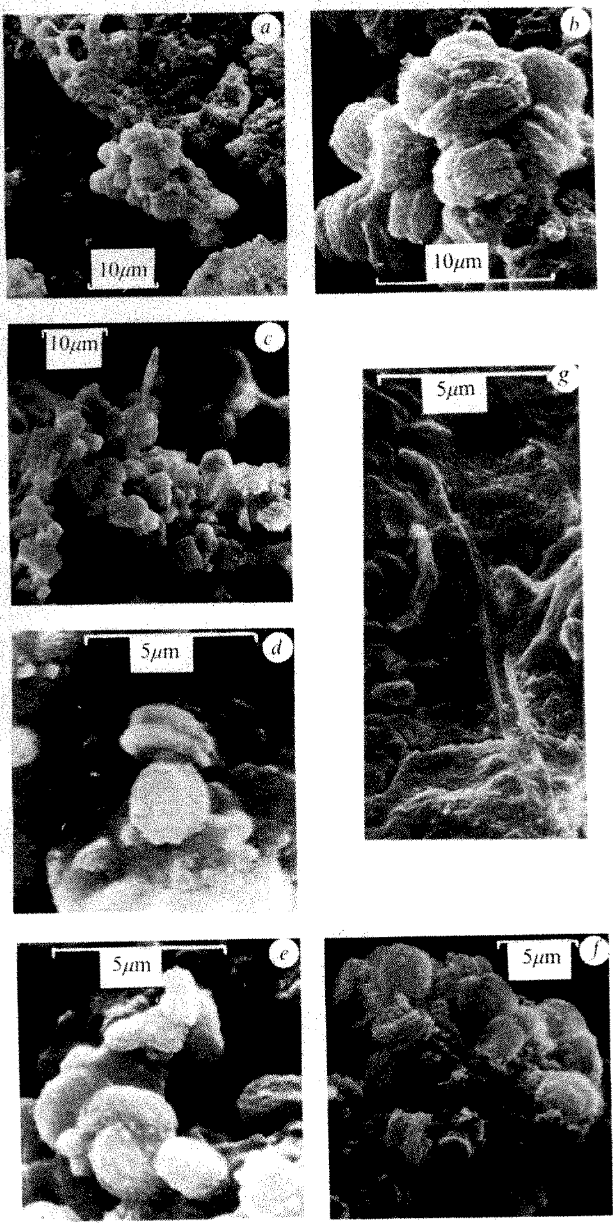

Fig. 2 *a–f*, Rounded microfossils (*b* is a close-up of *a*); *g*, filaments (composite micrograph).

sample. The samples were short centrifuged and washed until free of acid, and were then treated with 38–40% hydrofluoric acid for 6 h, centrifuging down the residues and changing the acid every 2 h. The final residues were short centrifuged and washed until free of acid, and the stub tubes were left to dry overnight in a clean vacuum desiccator. The following day, the residues were prepared[7] for SEM observations. Control preparations of all reagents were examined by SEM, revealing many artefacts (Fig. 1*a–c*). Laboratory distilled water was evaporated to form an ornamented crust (Fig. 1*a*), and de-ionised water carried over microspheres of resin (Fig. 1*b*). So to wash the residues, glass distilled water was de-ionised and then distilled again in a still cleaned with chromic acid. To determine whether pure silica could produce organised microstructures after reaction with hydrofluoric acid, a silica glass test tube was sacrificed, and when treated in the same way as the rock, structures were produced (Fig. 1*c*).

Examination of rock residues in the SEM revealed considerable amounts of organic and mineral debris, together with organised microstructures. Figure 1*d* probably represents a precipitate of fluorides[8], and we believe the structures of Fig. 2*a–g* to be the remains of microorganisms, a judgement based on their superficial resemblance to extant genera of algae[9] and other described fossil forms[10]. We consider it unlikely that these structures were produced by inorganic precipitation or by recrystallisation.

We believe that these preparations are of technical interest in that they demonstrate the feasibility of using the SEM wax stub tube for micropalaeontological maceration when it is necessary to reduce contamination to a minimum. We have also found the stub tube invaluable in monitoring the artefact content of distilled water, and of active reagents such as hydrofluoric acid. Although we do not suggest that the stub tube should replace other mounting techniques in scanning electron microscopy, we submit that it can be a useful tool in the critical study of organic microfossils.

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3,500-Myr-old granite in southern Africa

A GEOCHRONOLOGICAL study of the margin between the Rhodesian Craton and the Limpopo Belt in southern Africa has yielded the first conclusive evidence for the preservation of very old crustal material on the Rhodesian Craton. An age of 3,520±260 Myr has been obtained from a seven-point Rb–Sr whole rock isochron for samples of the Mushandike Granite, which intrudes tonalite gneisses near Fort Victoria (30° 50′E, 20° 04′S) in south-eastern Rhodesia. Its relation to the Bulawayan Group of the Victoria Schist Belt is uncertain. The possible presence of an intrusive contact of the granite within these rocks points out the need for a re-examination of the evidence for the widely accepted maximum age for the Bulawayan of 3,300–3,400 Myr (ref. 1).

The Mushandike Granite and its contacts with surrounding rocks have been described by Wilson[2,3]. For the most part, the 'granite' is a medium grained, massive granodiorite. It has undergone shearing and faulting since emplacement; the effects of that are especially intense in the southern and western parts, whereas in the northern and central parts little or no deformation has occurred. Evidence that the Mushandike Granite intrudes the Tonalite Gneiss Complex is strong. Wilson[2,3] reported xenoliths, typical of the

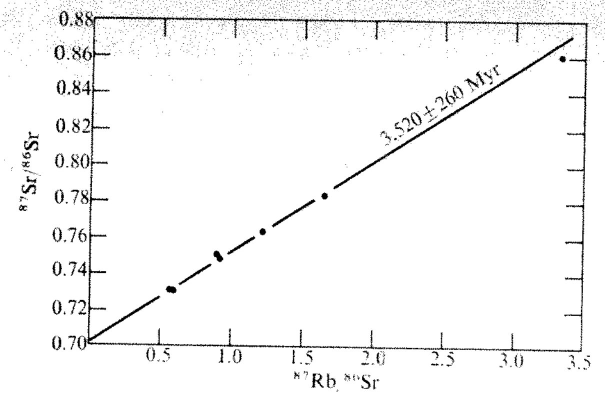

Fig. 1 Rb–Sr whole rock isochron for the Mushandike Granite.

surrounding gneisses, both in the centre of the granite and along its northern margin. In the north and south-east, the granite forms a lit-par-lit contact zone with the Gneiss Complex. Banding in the gneisses is roughly parallel to the contact along both margins, but tongues of granite cut across the banding in the north[3].

Along the southern margin of the granite, phyllites and amphibolites, assigned to the Bulawayan Group of the Victoria Schist Belt[2,3], occur. Shearing and faulting have obscured the original nature of the contact. Wilson[2,3] considers the granite to be post-Bulawayan. A narrow tongue of deformed granite, which can be traced into recognisable Mushandike Granite, occurs in the schist belt, suggesting an intrusive relationship. In one location the contract cuts across the trend of the phyllites. The occurrence of randomly oriented andalusite in the phyllites, and of cordierite and anthophyllite in the amphibolite, could be a result of contact metamorphism by the granite. Without a better understanding of the effects of the intense deformation in the contact zone, this evidence cannot be taken as conclusive; the present contacts could all be tectonic.

The Rb–Sr whole rock isochron (λ $^{87}Rb = 1.39 \times 10^{-11}$ yr^{-1}) for the Mushandike Granite, gives an age of 3,520 ± 260 Myr (Fig. 1); the analytical data are shown in Table 1. The seven analysed samples are all from the centre of the granite, away from the effects of deformation. There is evidence of some geological error in the data, and the quoted error in the age is that given by the isochron calculations McIntyre II and York I (ref. 4). Errors are quoted at the 95% confidence level.

Table 1 Analytical data

No.	Rb (p.p.m.)	Sr (p.p.m.)	$^{87}Rb/^{86}Sr$	$^{87}Sr/^{76}Sr$
1	71.8	354	0.587	0.7307
2	69.2	340	0.590	0.7305
3	65.5	209	0.907	0.7504
4	86.3	268	0.934	0.7487
5	63.9	151	1.22	0.7635
6	111	194	1.65	0.7840
7	107	93.9	3.32	0.8611

The calculated age is important in establishing conclusively the preservation of ancient crustal material on the Rhodesian Craton. It supports the findings of Robertson[5] who, on the basis of a detailed study of Rhodesian leads, postulated a ~3,500 Myr event on the Rhodesian Craton. He suggested that the event was associated with the formation of granitic crustal segments as well as with pre-Bulawayan lavas.

On the basis of field relationships, the Tonalite Gneiss Complex must be older than the Mushandike Granite. Preliminary isotope data for tonalite gneisses from the town of Fort Victoria, 10–15 km to the east suggest, however, that they may not record a significantly older isotopic age. The points do not define an isochron, showing considerable scatter, but they show no systematic deviation relative to the Mushandike Granite isochron. That may mean that correlations in the Gneiss Complex cannot be made over the distance involved, or that the intrusion of the Mushandike Granite was part of a widespread event which overprinted any isotopic record of the early history of the gneisses in the area. It is also possible that the emplacement of large bodies of granite north and south of the Victoria Schist Belt about 2,650 Myr ago[1] may have caused some isotopic mixing in the Fort Victoria gneisses at that time.

The ambiguity of the contact between the Bulawayan Group of the Victoria Schist Belt and the Mushandike Granite points out the need for reliable and precise age determinations on material from the schist belts themselves and on granitic bodies which have reasonably clear relationships to the schist belts. The ambiguous nature of the Mushandike–Bulawayan contact and uncertainty in the available, relevant age data mean that the age of the Bulawayan is now in question. The work of Robertson[5] suggests that most of the volcanics assigned to the Bulawayan Group are younger than 3,500 Myr, because leads recording a ~3,500 Myr event are generally restricted to pre-Bulawayan rocks. Three K–Ar determinations on muscovite from the pre-Bulawayan Que-Que Granite, about 160 km north north-west of Fort Victoria (29° 48′E, 18° 53′S) have given an age of 3,300–3,400 Myr (ref. 1). Although this clearly needs to be confirmed by other dating methods, it has, in the absence of other data, been widely accepted as an approximate maximum age for the Bulawayan.

Elsewhere in southern Africa, rocks older than 3,000 Myr have been found in the Barberton Mountain Land of the Kaapvaal Craton[6]. Of particular interest here is the new evidence, from Rb–Sr analysis of mineral fractions, that basalts of the Lower Onverwacht Group have an age of 3,500 ± 200 Myr. These rocks have been correlated with pre-Bulawayan volcanics on the Rhodesian Craton[7]. The Ancient Tonalite Gneisses which intrude the Lower Onverwacht have a Rb–Sr whole rock age of 3,440 ± 300 Myr (ref. 8).

No definite conclusions about the Rhodesian Craton can be drawn on the basis of Kaapvaal Craton age data. Chronological equivalence cannot be inferred from lithostratographic correlations between cratons, or even between different regions of a single craton. Until further work is done on Rhodesian material, the chronology of the Bulawayan Group remains uncertain.

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Thermal model for origin of granitic batholiths

THE origin of granitic batholiths has been reviewed in the light of our understanding of the processes operative at destructive plate margins[1]. The thermal patterns at island arcs suggest that significant amounts of thermal energy must be released within downgoing oceanic plates, and that this energy may be transferred to the surface behind island arcs. In these regions heat flow values are abnormally high and andesitic volcanism is present. The high heat flow may be produced because the normal geothermal gradient is enhanced by penetrative convection of magmatic material which is derived from the downgoing plate. Many large granitic batholiths, such as those in Chile, are localised at plate margins, and some authors have proposed that the Sierra Nevada batholith originated in a similar setting along the continental margin in the downwarped portion of the lower crust. Dickenson[2] suggested that the magma for the batholiths was produced by partial melting within the downgoing plate, and was subsequently emplaced into the overlying continental crust. Bateman and Dodge[3], and Brown[1] suggested that emplacement into the lower crust, of andesitic material derived from the mantle, causes partial melting of crustal material, with the mixed product forming the magma for the granitic batholiths.

Experimental studies of the generation of granitic melts[4,5] from metamorphic rocks have shown that melts of granitic composition will occur under conditions of crustal pressure at temperatures of about 700–750° C provided that water is released by dehydration reactions. A granitic melt may be produced by partial melting of the crust at depths of 25–30 km, whereas granodiorites and diorites may be derived from partial melting at higher temperatures (800–950° C) and deeper crustal levels (30–45 km)[1]. At the continental margins where the crust is 40–50 km thick, the radioactive content and the thermal conductivity of crustal rocks, together with the heat flow values, suggest that the lower crust is at a temperature of around 700° C, although higher temperatures may be achieved locally, adjacent to mantle-derived magma. Using thermal considerations I have investigated the extent of partial melting in sialic crust adjacent to a deep seated intrusion.

The thermal effects which a magma induces in country rocks are considered first. The cooling of a magmatic body that is assumed to be instantaneously emplaced may be expressed by the time-dependent equation for heat transport in one dimension:

$$K(\delta^2 T/\delta Z^2) = \rho c_p(\delta T/\delta t) \qquad (1)$$

where K is the thermal conductivity, ρ is the density, and c_p is the specific heat. Solutions to the equation extended to handle partial melting, can be approximated by finite difference techniques: the governing difference equation solved for temperature is

$$T(Z, t+\Delta t) = [1 - 2K\Delta t/\rho c_p(\Delta Z)^2]\,T(Z,t) + K\Delta t/\rho c_p(\Delta Z)^2$$
$$[T(Z-\Delta Z, t) + T(Z+\Delta Z, t)] \qquad (2)$$

where $T(Z, t+\Delta t)$ is the temperature at time $t+\Delta t$ at a location Z; Δt is the time increment; and ΔZ is the distance increment. The finite difference approximation of the heat conduction equation for two different media, country rock and magma, requires that the respective values of ρ, K and c_p for each medium are used in equation (2). A boundary layer equation is then adapted for the contact between country rock and magma.

I have modified the numerical techniques to handle partial melting. Partial melting is typically an endothermic process, and the total amount of heat of fusion, H, necessary to melt a material is a function of the rock composition. The total melting of basalt may absorb 400 calorie g^{-1}, and 10% partial melting may absorb, proportionately, 40 calorie g^{-1}. The latent heat of fusion of rocks of granitic composition is about 50 calorie g^{-1} (ref. 6). Thus, if it is assumed that a metamorphic rock is composed of 50% granitic material with the other 50% of mafic composition, to melt the granitic fraction of this rock would require a minimum of 25 calorie g^{-1}. It can be assumed that H is absorbed linearly over a range of melting, or as assumed here, the melting process can be simulated by absorption of H at a constant temperature, similar to the melting of a single component chemical species. Although the melting of a metamorphic rock is probably more complicated, that assumption is probably a reasonable description of the fusion process[7].

If a point, Z, reaches the temperature of melting, TM, it absorbs heat according to the equation

$$\Delta H = c_p \Delta T$$
$$\text{where } \Delta T = T(Z, t+\Delta t) - TM. \qquad (3)$$

The temperature of the point, Z, is TM until the total heat of fusion, H, has been absorbed. Once H is absorbed, the point responds according to equation (2). The absorbed heat will be released—equation (3)—as the temperature falls to TM and solidification begins; the heat of solidification is assumed to be equal to H. Conservation of energy is maintained throughout the process.

A simple geological model may best illustrate the effect of partial melting in country rocks adjacent to a magmatic body in the lower crust. The following parameters are used: $TM = 700°$ C; ρm (density of magma) $= 2.7$ g cm^{-3}; ρc (den-

Table 1 Partial melt zones around emplaced magma

Width of magma (km)	Initial temperature of country rock (° C)	TM of country rock (° C)	H (calorie g^{-1})	Total width (both sides) of zone of partial melting (km)
2	500	700	50	0.8
4	500	700	50	1.6
6	300	700	50	1.2
6 (no convection)	400	700	50	0.9
6	400	700	50	1.8
6	500	700	50	3.0
6	500	700	100	2.4
6	500	800	50	1.2
6	500	800	100	1.0
6	600	700	50	5.2
6	600	800	50	2.2
10	500	700	50	4.0

sity of country rock) = 3.3 g cm^{-3}; Km = 0.01 calorie cm^{-1} s^{-1}; Kc = 0.007 calorie cm^{-1} s^{-1}; T_m = 1,200° C (temperature of magma); H = 50 calorie g^{-1}; m = magma; c = country rock. Consider a slab shaped magma, 4 km thick, emplaced into a lower crust with an original uniform temperature of 500° C. It is likely that a mafic magma of this size, and a viscosity of 10^6 poise, will convect naturally[8]. One of the effects of convection within the magma will be to raise the temperatures in the country rocks above the maximum which would have been achieved if the magma was cooling without convection (Table 1). It is assumed that the temperature distribution within a freely convecting magma will be governed by the adiabatic law so that the temperature may vary with depth by anything from 0.3 to 1° C km^{-1}. Therefore the change in temperature from the top to the bottom of a convecting body 4 km thick will be only 2° to 4° C. The temperature distribution within and outside the magma at different times, for one half of a symmetrical model, is shown in Fig. 1. The temperatures reflect the heat (H = 50 calorie g^{-1}) which is absorbed in the country rock as a result of melting once the melting temperature, TM (700° C) has been reached. After 40,000 yr, 0.8 km of country rock would be partially melted and the total partial melt zone on both sides of the magma chamber would be 1.6 km wide.

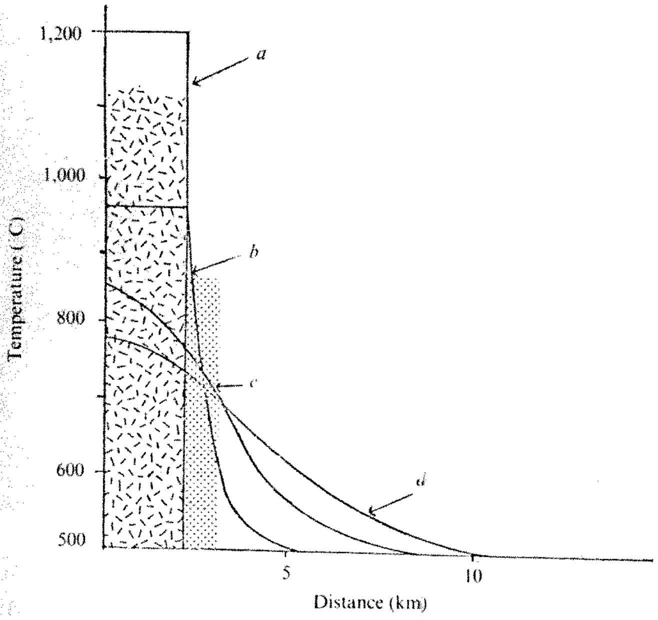

Fig. 1 The temperatures around a symmetrical magma 4 km thick: *a*, at emplacement; *b*, after 2 × 10^4 yr; *c*, after 10^5 yr; *d*, after 2 × 10^5 yr. Stippled area is the zone of partial melting. TM = 700° C; H = 50 calorie g^{-1}; initial temperature of the magma = 1,200° C. At T_m = 900° C convection ceases and the magma cools by conduction.

As most magmas derived from the mantle will have properties that do not vary greatly from those assumed here, the size of the partially melted zone around an intrusive will be principally a function of the thickness of the intrusive and of the initial temperature and composition of the country rock. Table 1 shows the width of zones of partial melting for several different examples. In each of these examples the parameters had the values already given here, and except in the cases specified in Table 1, convection within the magma was assumed.

Using H = 50 calorie g^{-1}, a magma 6 km thick intruded into country rock which is at 600° C, would partially melt a zone 2.6 km thick on each side. Within the zone of partial melting the amount of liquid granitic melt is a direct function of the original composition of the metamorphic rock. Thus, for example, with a metamorphic rock which was originally 50% granitic and 50% mafic, the zone of partial melt may be 50% liquid. A slab of magma 6 km thick, emplaced into country rock which is at 400° C, would have a zone of partial melting with a total combined width on both sides, of 1.8 km. If a magma were emplaced into a metamorphic rock of mafic composition with little granitic fraction, the width of the zone of partial melting would be reduced. This can be illustrated by taking H equal to 100 calorie g^{-1} instead of 50 calorie g^{-1}. The width of the zone of partial melting is reduced from 3.0 to 2.4 km thick (Table 1), but the amount of partial melting is still substantial. For a case in which the country rock has a higher temperature of melting (TM = 800° C) and a larger heat of fusion (H = 100 calorie g^{-1}) than the magma, the total width of the zone of partial melting on both sides of the magma would be only 1.0 km. As these properties may be more similar to a mafic metamorphic rock, the partial melting of metamorphic rock around a magma is probably limited. These results (Table 1) indicate that large portions of country rock with some fraction of granitic composition, can be melted under less extreme crustal conditions, and magma convection greatly increases the extent of partial melting in country rocks. Similar quantities of magma will produce, however, little melt in the upper crust (300° C).

If a substantial proportion of the zone of partial melting is liquid, then that liquid may mix with the magma. The amount of physical mixing between magma and country rock is partly dependent upon the relationship between the amount of time it takes the magma to solidify, and the time during which the country rock is melted. At the outer front of the zone of partial melting the country rock may still be melting after the mafic has cooled below its temperature of solidification. As long as the magma is above the solidus, however, the zone of partial melting in the country rock could mix with the magma and thus alter its composition. As an example, it can be assumed that solidification of the magma occurs at 900° C. Then an intrusive 6 km thick within country rock which had an original temperature of 500° C, will solidify after about 92,000 yr (Fig. 2). By that time the country rock would be partially melted out to 1.2 km from the contact, and most of the melt could mix with the magma before it solidified. If only a portion of the country rock melted, then limited amounts of granitic melt above and below the contact would be available for mixing. The refractory country rock material that does not melt may fall to the floor of the magma or be incorporated within it. In this manner the original magma could be diluted with both fused granitic material and refractory country rock.

The extent of partial melting around a slab shaped magma emplaced into a crust with a moderate geothermal gradient, is

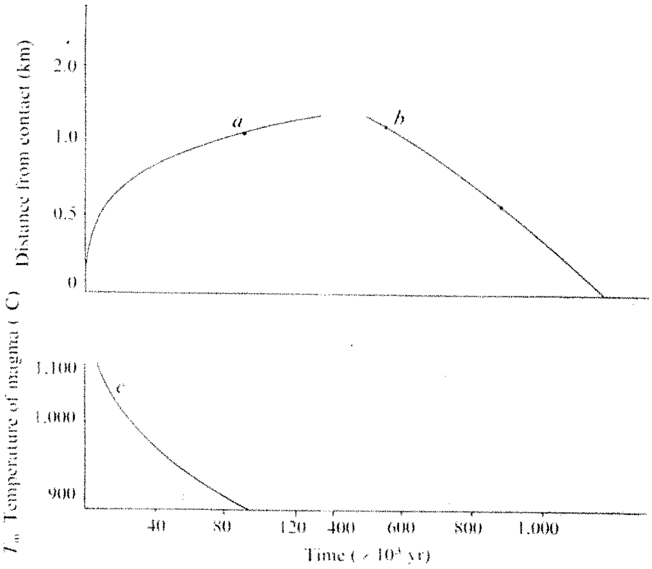

Fig. 2 *a*, The time which elapses between emplacement of magma and the onset of partial melting at increasing distances from the contact; *b*, the time which elapses between emplacement and the onset of solidification in the zone of partial melting at varying distances from the contact; *c*, the rate of cooling of a convecting magma.

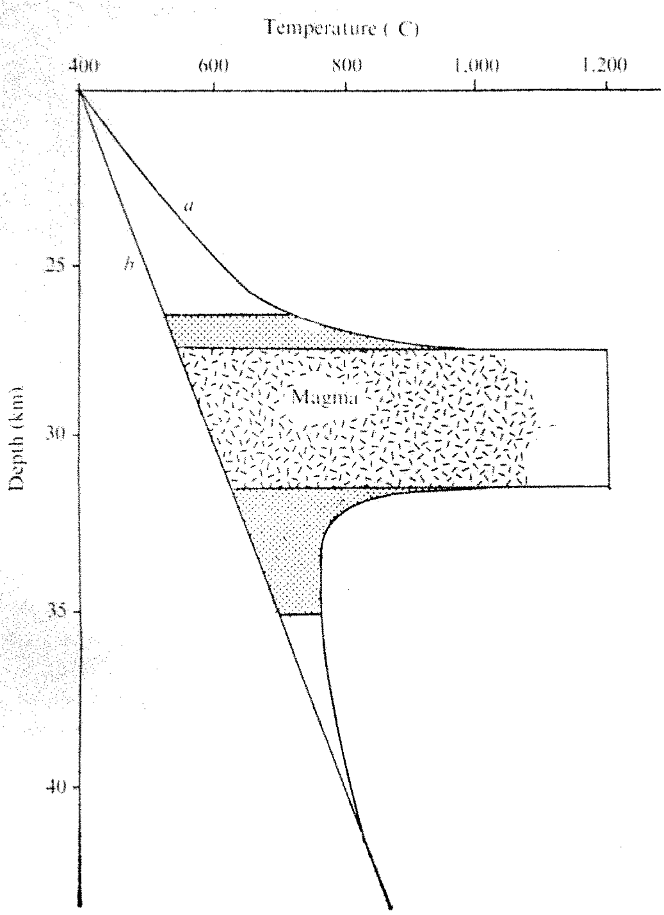

Fig. 3 Temperatures around an intrusive which is 4 km thick, and centred on a depth of 30 km: *a*, maximum temperatures; *b*, geothermal gradient; stippling, zone of partial melting. Initial temperature of magma = 1,200° C; H_f = 50 calorie g^{-1}.

significantly different from that indicated by the model in Fig. 1. An asymmetrical orientation of the heat fluxing from the top and the bottom of the magma is produced. Taking an initial geothermal gradient of 20° C km^{-1}, and a magma slab 4 km thick emplaced at 30 km depth, the country rock temperatures below the magma would be at least 80° C more than above the magma, and those temperatures would increase with depth (Fig. 3). In this thermal model the values of the parameter given already define the initial boundary conditions, and crustal rocks initially hotter than 700° C (35 km depth) are assumed not to undergo partial melting. The maximum temperatures achieved in the country rock in a case such as that are shown in Fig. 3. About 1 km of country rock above the upper contact, and about 4 km below the magma, would undergo partial melting. The total width of the zone of partial melting would exceed the initial thickness of the magma. In that case, the total heat flux from the top of the magma would be about twice that from the bottom. As much as 5 km of the country rock would have been melted partially, whereas in the model shown in Fig. 1, the partially melted zone is only 1.6 km thick adjacent to a slab of magma of the same size, with initial country rock temperatures of 500° C.

The process of partial melting and solidification of the country rock adjacent to an intrusive, takes approximately 2×10^5 yr in the cases discussed. Solidification of the mafic parent rocks at 900° C takes about 10^4–10^5 yr. Thus, the zones of partial melting adjacent to a magma may have a higher mobility for a considerably longer period of time than either the solidified magma or the lower crustal material that has not undergone partial melting. These zones of partial melting may serve as sites for the emplacement of other material derived from the mantle, and further partial melting in the lower crust may follow.

These thermal models suggest that there are a number of possible ways in which an intermediate rock type could be derived from the mixing of a mantle derived parent with partially melted lower crustal rocks that are adjacent to the intrusion. Dense refractory material in the zone of partial melt, as well as in the parent magma, will tend to sink and lighter granitic fractions will tend to migrate upward. Granitic material may be formed from the partial melt zones above and below the magma and from differentiation of the original magma. These lighter granitic liquids could accumulate into large magmas in the lower crust and then migrate and collect to form plutons of batholithic proportions in the upper crust. In this two stage model of the origin of granitic batholiths along continental margins, the production of the intermediate granitic rocks may be intimately associated with the production and migration of magmas of andesitic to basaltic composition from active subduction zones at the continental margins.

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Thermal contraction joints in a spreading seafloor as origin of fracture zones

If newly formed oceanic crust is moving away from a spreading axis, it can be expected to contract by cooling. Vertically, the cooling is expressed in the existence and cross-sectional shape of the mid ocean ridges, where there is a relationship between depth of the ocean and age of the crust[1]. A steady state solution for a model of a lithospheric plate which cools while moving[2–4] is compatible with data on topographical height.

Horizontally, in the direction of spreading, cooling will result in the formation of superficial horst and graben structures parallel to the median rift[5,6]. Also in the third direction—that of the ridge axis—internal stresses will arise which are much larger than the breaking strength of rock. If the thermal expansion coefficient λ, is 1.5×10^{-5} ° C^{-1}, and Young's modulus, *E*, is 1×10^6 kg cm^{-2}, a decrease in temperature of 1,000° C gives an internal tension of 15,000 kg cm^{-2}. Faulting can therefore be expected to occur perpendicular to the direction of the ridge axis, and I propose that fracture zones are the topographical expression of those faults (Fig. 1 and refs 7–10).

The hypothesis provides a model for an orthogonal median rift–fracture zone system[11]. The actual shape of the ridge as a whole will be dictated by other causes, such as the shape of the initial rift. That is exemplified by the parting of North America and South America from Africa. A change in spreading direction can also place constraints on the shape of the ridge. Straight ridges with fracture zones and no offset could be explained using this model:

the thermal contraction character is primary, and the transform fault function[13] is secondary.

Additional evidence of thermal contraction in the direction of the ridge axis comes from the distribution of fracture zones in the central North Atlantic and the equatorial Atlantic. There, the Mid-Atlantic Ridge is far from straight. The active sections of the fracture zones do not obey strictly the small circle pattern dictated by purely geometric or kinematic considerations[14]. The departures from that pattern are significant and apparently systematic (Fig. 2). Fracture zones and median rift segments still form an orthogonal system but the pattern as a whole becomes fanned, following to some degree the bends of the ridge.

The segments of the median rift and the fracture zones therefore form a complex dovetail construction, interlocking opposing lithospheric plates. Thus, in order to free the plates, about 6% of shortening in the direction of the ridge axis is needed. That is of the right order of magnitude compared with the maximum depth of the ocean, which in these parts varies between 5.5 and 6 km. That would lead to a total vertical contraction of 3–6%, depending on the thickness assumed for the lithosphere.

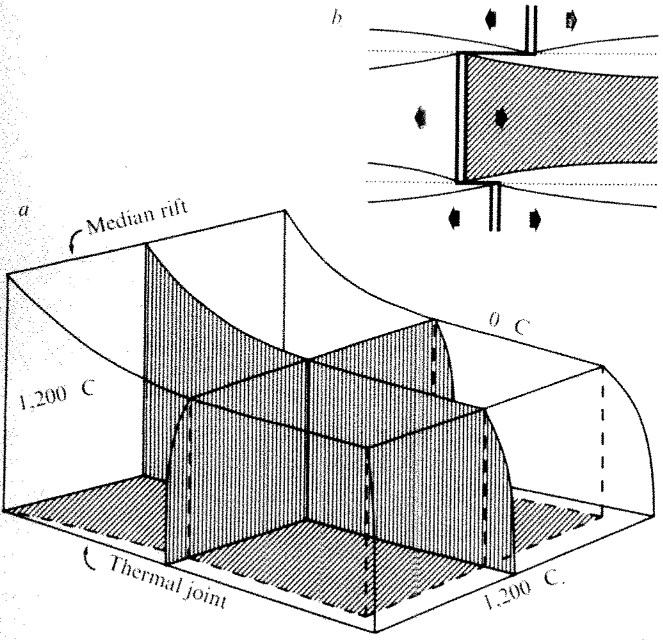

Fig. 1 *a*, Seafloor spreading implies cooling of newly formed lithosphere. A plate moving from the median rift will contract as shown. Thermal joints develop at right angles to the ridge axis. *b*, Plan view of thermal joints accommodating transform faults; arrows indicate spreading directions.

The fanning of fracture zones can be understood by considering the mechanism which makes the plates move. Thinking in terms of gravitational sliding, the analogy to glacier flow[15] may be extended by comparing the 'bights' of the Mid-Atlantic Ridge to a firn or névé: the shape of the bights would determine the flow pattern of the lithosphere which slides passively downwards to both sides, coagulating into rigid plates in which the stress trajectories are roughly parallel to the fossil fracture zones and to the growth lines of the lithosphere, that is, the magnetic lineations. The stress trajectories would then converge off the concave sides of the ridge, thus accounting for the fanning. A pull, exerted by the sinking lithosphere in the subduction zones and/or by a friction exerted by the upper mantle at the base of the lithosphere may be added to this. If the latter effect were dominant, however, fanning still occurs because the ridge represents a curvilinear zone of weakness in the lithosphere. A zone such as this (with a lower Young's modulus) in an elastic plate under tension would be reflected in the stress distribution. Experiments with a sheet of pararubber have shown that the longer axes of the strain ellipsoids are deflected towards the normal to the weakened zone.

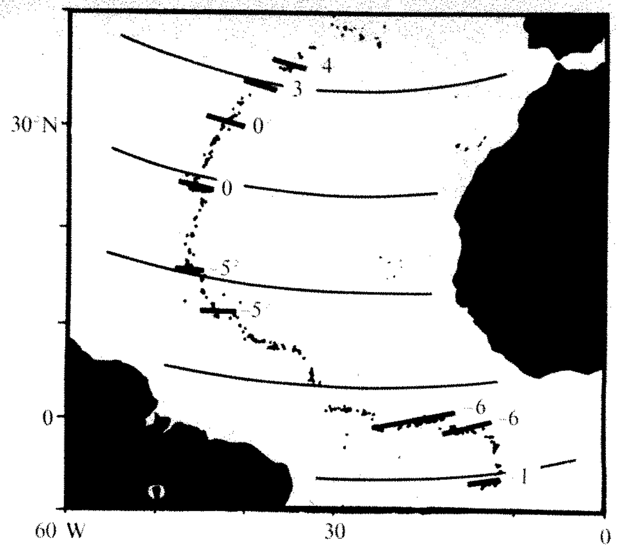

Fig. 2 Departure of fracture zone azimuths from a small circle pattern with a pole spreading at 63°N and 27°W.

Once the pattern has been established and the fracture zones begin to function as transform faults, the stress distribution will be affected if some form of welding occurs along the plane of the fracture zone. Extrapolation of the results of the experiments with pararubber, indicates that the larger principal stress may be expected to become deflected towards the normal to the fracture zone, thus releasing the normal stress in the plane of the fracture zone, and easing the movement.

The observation that fracture zones may be fanning implies that, although statistically and on a global scale the small circle concept[14,16] remains valid, poles derived from individual fracture zones or from a number of close fracture zones do not necessarily represent the true spreading pole. True spreading poles can only be found by averaging weighted azimuths of all of the fracture zones in a given area. The degree of fanning is also a function of age, with the fanning reaching its maximum at the ridge axis. The present data on the central North and equatorial Atlantic do not yet allow a final analysis.

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called satellite I, has been estimated to be 6–7% of the total DNA[16,17]. Here I report studies with restriction endonucleases which show that this satellite DNA has a repeat unit which is 1,400 (± 50) bases long. This higher order repeat unit which accounts for more than 95% of the satellite is internally repetitious.

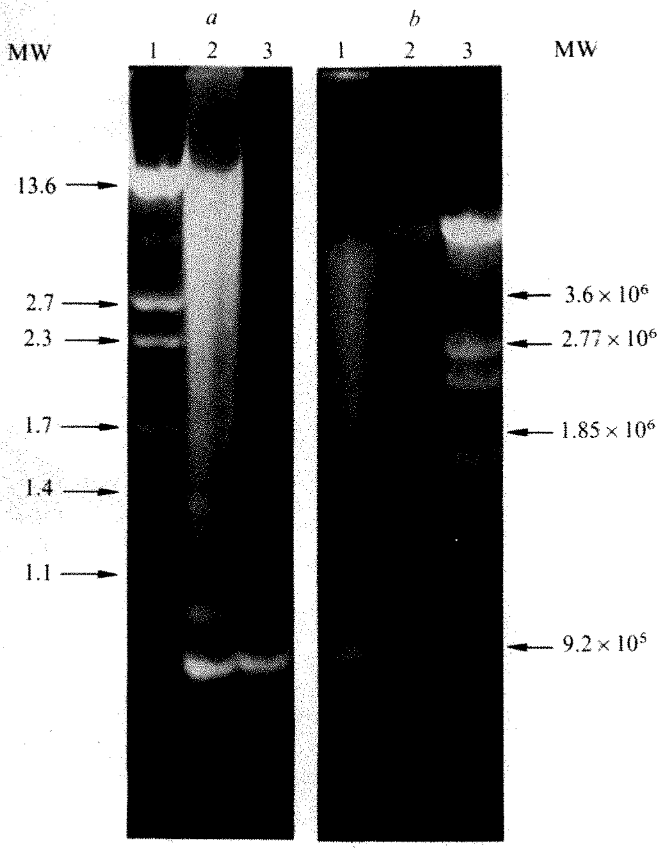

Fig. 1 *a*, Endonuclease EcoRI cleavage of total and satellite I bovine DNA. Bovine kidney cells (MDBK) were grown in Dulbecco's media containing 10% calf serum, and 5 μCi ml^{-1} of ^{32}P as orthophosphate. Confluent monolayers of cells were collected and the DNA extracted as described previously[35]. Cells were lysed in 0.5% SDS and subjected to a pronase (1 mg ml^{-1}) digestion for 8 h in 0.01 M Tris *p*H 7.9 + 0.001 M EDTA. The lysate was extracted with phenol and chloroform-isoamyl-alcohol (24:1 v/v) and then dialysed extensively. After digestion with RNase (100 μg ml^{-1}) and redigestion with pronase, the DNA was re-extracted with phenol. The DNA was then dialysed into 0.01 M Tris *p*H 7.9 + 0.001 M EDTA. The DNA extracted by this procedure had a specific activity of 19,700 c.p.m. μg^{-1}. 100 μg of total DNA was placed in a 5 ml solution of CsCl (density 1.700 g ml^{-1}) and centrifuged for 48 h at 50,000 r.p.m. and then 20 h at 40,000 r.p.m. Fractions corresponding to satellite I DNA were pooled and dialysed into 0.005 M $Na_2B_4O_7$ + 0.005 M Na_2SO_4 *p*H 8.9. $AgNO_3$ was added to an f of 0.40 and the solution brought to a density of 1.500 g ml^{-1} with Cs_2SO_4. The satellite DNA was again banded in this solution by centrifugation as described above. The fractions of the major peak (corresponding to 80% of the DNA in the gradient) were pooled. (1) 2 μg of Ad2 virus DNA, (2) 2 μg total bovine DNA, (3) 1 μg satellite I DNA were digested separately for 2 h in 0.01 M Tris *p*H 7.9 + 0.01 M $MgCl_2$ at 37° C, with EcoRI. The volume of the reaction mixture was 100 ml and contained 0.03 mg ml^{-1} of the restriction enzyme. The enzyme was isolated as described[21,22,36]. The reaction mixtures were adjusted to 20% sucrose and layered onto a 1.5% agarose slab gel. After electrophoresis in E buffer[22,36] at 5 V cm^{-1} for 10 h, the DNA in the gel was stained with ethidium bromide (0.5 μg ml^{-1}). The gel was photographed with ultraviolet excitation of ethidium fluorescence[36]. The molecular weights correspond to the Ad2 fragments A–F shown in (1)[22]. *b*, Partial digestion of satellite I DNA with EcoRI. 1 μg of high molecular weight satellite I DNA was digested with EcoRI as described in Fig. 1*a*, but the reaction was stopped after 20 min by adjusting the mixture to 0.01 M EDTA and 0.5% SDS. The reaction mixture was then subjected to electrophoresis as before. The partial digestion is in position (2). (1) is the total bovine DNA digested to completion and (3) is the Ad2 markers. The molecular weights shown refer to the partial cleavage products.

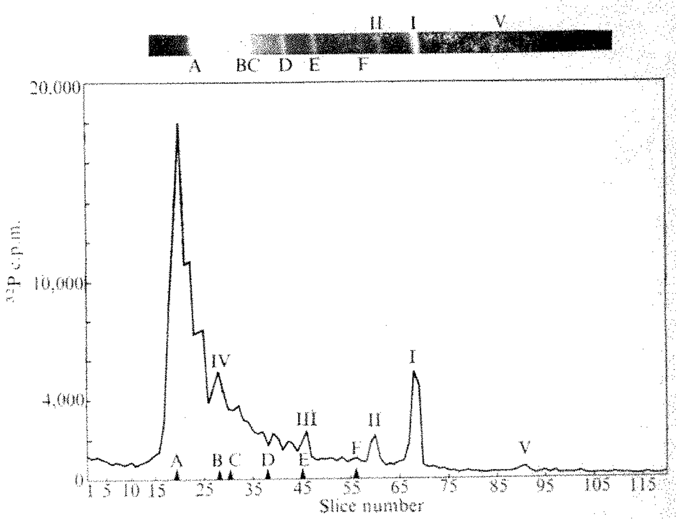

Fig. 2 Distribution of fragment sizes of total bovine DNA after digestion with EcoRI. 7.5 μg of ^{32}P-labelled bovine DNA (specific activity 1.9 × 10^4 c.p.m. μg^{-1}) + 0.3 μg ^3H Ad2 DNA (specific activity 10^6 μg^{-1}) were digested with EcoRI as described in Fig. 1. The reaction was stopped with 0.5% SDS + 0.01 M EDTA and extracted once with phenol. The DNA fragments were precipitated with ethanol and resuspended in 50 μl of one quarter XE buffer. The sample was layered on to a 0.5 cm × 12 cm cylindrical gel of 0.7% agarose–2.2% acrylamide[36] and electrophoresed for 12 h at 50 V. The gel was stained and photographed as in Fig. 1. The gel was sliced into 1 mm sections and each fraction placed into 3 ml of Aquasol (New England Nuclear). The slices were soaked overnight and counted. Arrows refer to peak positions of the Ad2 DNA markers. The amount of DNA in each peak I–V is given in the text and is the average of five such measurements.

Restriction endonuclease cleavage

Bovine kidney cells were grown in tissue culture and the total DNA was labelled and extracted as is described in the legend to Fig. 1. The DNA thus extracted was of high molecular weight as the mean sedimentation coefficient as measured by centrifugation in a neutral sucrose gradient was 60S. Satellite I DNA has a density of 1.716 g ml^{-1} (refs 16, 17) in neutral CsCl and was isolated from the bulk of the DNA, which has a mean density in CsCl of 1.700, by both Ag^+–Cs_2SO_4 and CsCl equilibrium centrifugation techniques[16–19]. After the isolation of this satellite, the DNA was digested to completion with the restriction enzyme EcoRI (see ref. 20 for nomenclature of restriction enzymes), which cleaves DNA at the sequence

$$\begin{matrix} \downarrow \\ {}^{5'}\mathrm{GAATTC}^{3'} \\ {}^{3'}\mathrm{CTTAAG}^{5'} \\ \uparrow \end{matrix}$$

(ref. 21). The products of this digestion were then analysed by electrophoresis through 1.4% agarose gels. Figure 1*a* is a photograph of a slab gel after electrophoresis and staining of the DNA with ethidium bromide and it illustrates that the material migrates in one discrete band. More than 95% of the radioactivity in satellite I DNA is recovered in this band. The molecular weight of this band of DNA was calculated to be 9.2 × 10^5 (± 6%), from its relative mobility to the EcoRI fragments of adenovirus 2 DNA[22] (Fig. 1). The molecular weight of EcoRI cleaved satellite I DNA indicates that within this DNA at points every 1,400 base pairs is the sequence $\frac{\mathrm{GAATTC}}{\mathrm{CTTAAG}}$. A partial digestion of the satellite DNA confirms the tandem nature of this repeat. If the enzyme digestion is stopped before completion, multiple bands of DNA are seen after electrophoresis whose molecular weights form an arithmetical progression (Fig. 1*b*). The monomer,

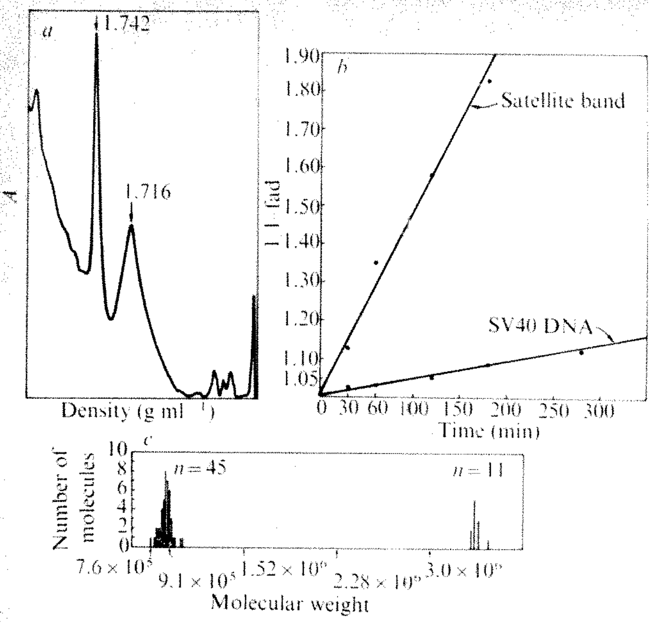

Fig. 3 Physical characterisation of satellite I DNA. *a*, Total bovine ^{32}P DNA was digested with EcoRI and electrophoresed as in Fig. 2. Fractions corresponding to peak I (fractions 66–69) were pooled and the DNA eluted by electrophoresis as described in refs 35 and 36. 0.5 μg of this DNA was centrifuged to equilibrium in CsCl at 44,000 r.p.m. in a Spinco Model E centrifuge. A densitometer trace of the *A* profile is shown. The density of 1.716 g ml^{-1} was calculated relative to SPOI DNA $\rho = 1.742$ g ml^{-1} using $1/\beta = 8.4 \times 10^{-10}$ c.g.s. units. *b* Renaturation kinetics of this ^{32}P satellite DNA were measured relative to ^{3}H-SV40 DNA by hydroxylapatite chromatography (Bio-Rad HTP) fad = fraction of DNA binding to HAP as double strands. 0.2 μg of the ^{32}P satellite DNA (specific activity 11,000 c.p.m. μg^{-1}) plus 0.42 μg of ^{3}H- SV40 DNA (40,000 c.p.m. μg^{-1}) were combined in 100 ml of 0.001 M Tris *p*H 7.9 + 0.025 M NaCl. 50 μl of 1 M NaOH were added and the solution boiled in a sealed capillary tube for 25 min to fragment the DNA. The solution was then neutralised with 50 μl of 1 M HCl and brought to 2.2 ml with 0.035 M phosphate buffer + 0.4% SDS. 0.5 ml were taken at the specified times and the fraction of molecules that were double-stranded was measured by chromatography at 60° C. Total eluants from the 0.14 M phosphate buffer and the 0.40 M phosphate buffer washes were precipitated separately with trichloroacetic acid and filtered on to Millipore HAWP filters. The ethanol washed filters were counted in Aquasol. The slopes of the lines differ by a factor of 11. Since the SV40 DNA is 2.1 times more concentrated than the satellite DNA, the kinetic rate constants differ by a factor of 23. *c*, Histogram of molecular weights of the EcoRI cleaved satellite I DNA: total high molecular weight satellite I DNA was digested with EcoRI as described in Fig. 1. The reaction mixture was extracted with phenol and the DNA precipitated with ethanol at −20° C. The precipitate was resuspended in 0.01 M Tris *p*H 8 buffer containing SV40 circular DNA. Molecular lengths were measured by electron microscopy as described in ref. 36. The lengths were converted to molecular weights by assigning a molecular weight of 3.4 × 10^{6} daltons for circular SV40 molecules.

dimer, trimer, tetramer and pentamer molecular weights of the 9.2 × 10^{5} dalton repeat were resolved.

The molecular weight of the EcoRI digested satellite DNA was measured by electron microscopy. A histogram of molecular weights is given in Fig. 3*c*. Assigning a molecular weight of 3.4 × 10^{6} for the circular SV40 DNA which was added as a marker, the EcoRI digested satellite had a molecular weight of 8.9 × 10^{5} (± 3%). There is then excellent agreement between the electron microscope and electrophoretic measurements of this repeat length.

Because satellite I DNA was cleaved to a uniform length by EcoRI, it seemed likely that cleavage with this restriction enzyme would provide a simple and quantitative procedure for the satellite isolation. The 1,400 base pair repeat in satellite I can indeed be detected in the EcoRI digestion products of the unfractionated total bovine DNA (Figs 1*a* and 2). When the total DNA is digested to completion with the endonuclease, a heterogeneous population of DNA molecules is obtained. Within this distribution one can clearly distinguish four bands of DNA, whose molecular weights are: 1.35 × 10^{6} (III); 10^{6} (II); 9.2 × 10^{5} (I); and 5.1 × 10^{5} (V) daltons (Fig. 1*a*). The source of the DNA did not influence these molecular weights as commercial calf thymus DNA (Worthington) yielded bands with DNA at identical molecular weights.

The amount of material in each of the bands was measured by digesting ^{32}P-labelled bovine DNA with EcoRI and analysing the fragments by electrophoresis. The distribution of ^{32}P-DNA, together with the positions of internal markers are shown in Fig. 2. The percentage of the total DNA under each band was calculated to be: 0.7% (IV), 0.5% (III), 1% (II), 6.7% (I), 0.1% (V) respectively for the peaks from highest to lowest molecular weight. The DNA from the peak labelled I was cut from a separate gel and eluted by electrophoresis. This DNA was identified as satellite I on the basis of the following criteria: (1) its buoyant density in a neutral CsCl gradient of 1.716 g ml^{-1} (see Fig. 3); (2) its relative amount in the genome of 6.7%; (3) its coelectrophoresis with EcoRI cleaved satellite I DNA; and (4) its renaturation kinetics being indistinguishable from that of satellite I (see below).

It is well known that the rate of reannealing of a unique sequence DNA is directly proportional to the complexity of the sequence[23,24]. Therefore, if the satellite I DNA were composed of a unique repeating unit 1,400 base pairs long, it should renature at a rate roughly four times faster than that of SV40 DNA, allowing for the fact that there is an 18% difference in G–C composition between the two DNAs. A comparison of the rates of renaturation of denatured SV40 DNA and denatured satellite I DNA is shown in Fig. 3*a*. The total kinetic complexity of the satellite as measured by renaturation kinetics is 200–250 base pairs. A complexity of 200 base pairs for the satellite I must mean that the 1,400 nucleotide repeat is internally repetitious. Based upon what is known about the structure of other satellites[11–14], a likely hypothesis is that the repeat unit has a short 8–20 base pair substructure with enough divergence in this basic sequence to cause an apparent complexity of 200–250 base pairs. The picture of this satellite then emerges as a repeating unit within a repeating unit. The EcoRI sequence repeat could be again part of a larger repeating unit containing the 1,400 base pair unit an integral number of times. In view of this, it was interesting to digest bovine satellite I DNA with another restriction enzyme.

At least two specific endonuclease activities can be isolated from *Haemophilus influenzae* serotype d. Hind II cleaves at the sequences

↓
$^{5'}$-GTPYPUAC-
-CAPUPYTA-$^{5'}$ (ref. 25). Hind III does not cleave satellite I
↑

(Fig. 4*b*). When high molecular weight satellite DNA is digested with Hind II or the combined enzymes, the DNA is degraded to two fragments of 7.4 × 10^{5} and 1.25 × 10^{5} daltons as measured by electrophoresis through 4% polyacrylamide gels (Fig. 4*a*). The same two bands can be seen in the Hind II + Hind III digest of total bovine DNA (Fig. 4*a*). The sum of the molecular weights of these two bands is 8.65 × 10^{5}. If the EcoRI repeat were part of a larger unit, two other enzymes might be expected to give fragments whose additive molecular weights indicated a greater complexity. There is a 3% difference between this estimate of the repeat length and the electron microscope length of the EcoRI repeat. A second enzyme system then indicates that there is a repeat unit every 1,400 base pairs within this satellite.

Restriction map of satellite

Within this repeat unit it is now possible to orient the EcoRI cleavage sequence and the two Hind II cleavage sites by comparing the products of digestion of the enzymes working

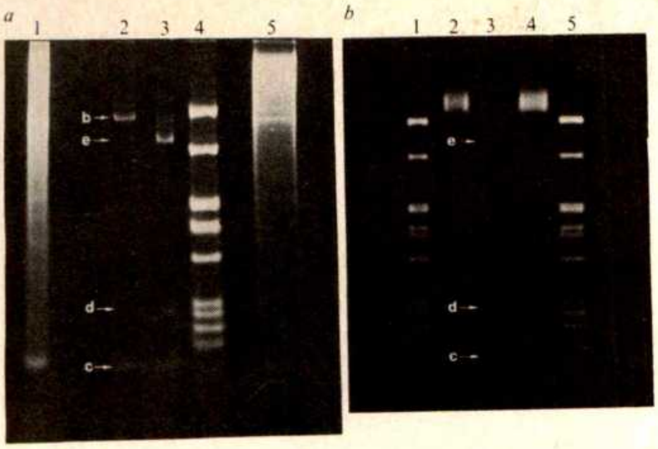

Fig. 4 Cleavage of satellite I DNA with Hind restriction enzymes. *a*, (1) total bovine DNA (1 μg) digested with Hind II + III restriction enzymes. (2) High molecular weight satellite I DNA (0.5 μg) digested with Hind II + III. (3) Satellite I DNA (0.5 μg) cleaved with EcoRI then with Hind II + III. (4) SV40 DNA (2 μg) cleaved with Hind II + III. (5) Total bovine DNA cleaved with Hind II + III. The Hind enzymes were isolated as described in ref. 25. The DNAs were cleaved in buffer containing enzyme and 0.006 M $MgCl_2$ + 0.01 M Tris *p*H 7.4 + 0.1 mM DTT for 12 h at 37° C. After digestion and extraction with phenol, the DNA was precipitated with ethanol and resuspended in 20 μl of 1/4 × E buffer containing 20% sucrose. The samples were then placed on to the 4% polyacrylamide slab gel. The gel contained 4% acrylamide (Bio-Rad), and 0.2% N′N′-methylene-bis-acrylamide (Eastman Kodak) and was polymerised by the addition of NNN′N′-tetraethylenediamine and ammonium persulphate. Electrophoresis was for 6 h at 50 V. Molecular weights were measured relative to the SV40 fragments[37]. b in position 2 = 7.4×10^5 daltons, c in positions 1,2,3,5 = 1.2×10^5 daltons, e in position 3 = 5.7×10^5 daltons, d in position 3 = 1.8×10^5 daltons. Other bands in 2 and 3 are partial cleavage products. *b*, EcoRI cleaved satellite I DNA was digested with Hind III (a gift of Dr R. Roberts). The Hind III + EcoRI cleaved DNA in position 2 has the same mobility as RI cleaved satellite in position 4. An aliquot of the Hind III reaction mixture was taken and to it added the Hind II enzyme. The DNA from this experiment is shown in position 3. The SV40 DNA fragments obtained by cleavage with Hind II + III are shown in positions 1 and 5.

separately and together on the DNA. Satellite I DNA was cut with EcoRI. After extraction with phenol, the DNA was redigested with the Hind II enzyme. The products of this double enzyme digestion were DNA fragments of three molecular weight classes as measured by electrophoresis in 4% polyacrylamide gels (Fig. 4*a*, *b*). The molecular weights are: 5.7×10^5, 1.8×10^5 and 1.2×10^5 daltons. That there is a true repeating unit within the satellite is further indicated by the finding that the amount of DNA in each one of these three bands is proportional to its fraction of the repeat length. Thus, the relative amounts of radioactivity in the three bands are 65%, 20% and 15%.

An unambiguous map of these restriction sites (see Fig. 5) within the repeat unit can be deduced from the following: (1) The distance between the two Hind II restriction sites is 1.2×10^5 daltons. This accounts for the 1.2×10^5 dalton fragment (c) seen in both the double enzyme (EcoRI + Hind II), and the Hind II digestions. (2) The RI cleavage site must be 1.8×10^5 daltons from one of the Hind II sites. This accounts for the 1.8×10^5 daltons band (d) resulting from the double digestion (EcoRI + Hind II). (3) The 7.4×10^5 dalton band (b) arising from the Hind II digestion is cut once by EcoRI to yield 5.7×10^5 (e) and 1.8×10^5 (d) dalton bands. These two bands orient the RI site with respect to the Hind II sites.

A conclusion of the restriction map shown in Fig. 5 is that the repeat unit detected is tandemly repeated (. . . ecdecdecd . . . .). Inverse tandem repeats (. . . ecddce . . .) can be eliminated as this would be detected as a band at 3.6×10^5 daltons (d + d) in the Hind II cleavage experiments.

Evolution of satellite I DNA

The evolution of satellite DNA poses a paradox. On the one hand, apparent fluctuations of satellite content in related species are quite well documented[5,12,26]. In the genus *Dipodomys* the amount of density satellite DNA ranges from barely detectable in *Dipodomys spectabilis*, to more than half the nuclear DNA content in *Dipodomys ordii*[27]. In general, it is not clear as to whether these sequences are lost from the basic genome or through translocations and mutations become unrecognisable. By contrast to this interspecies variability, is the remarkable homogeneity of repeats that can exist within one satellite[14]. Furthermore, it is clear that different satellites within the same organism[14,28] and in different species[14,29] are related to each other. The problem then in satellite evolution is to account for apparently rapid changes in content and in sequence, while allowing for homogeneity within tandem arrays and sequence relationships between different satellites.

Two basic mechanisms for satellite evolution can be considered, one being unequal crossing over and the other DNA replication. From mathematical considerations, Smith[30] has shown that if tandem arrays of sequences become heterogeneous, unequal crossing over will tend to homogenise the array. By this process of unequal crossing over, the sequence of one repeat unit will eventually dominate the cluster. With regard to the origin of arrays, unequal crossing over can also clearly increase or decrease the size of an array by a single recombination.

Mechanisms of satellite DNA evolution based on DNA replication must address themselves to the frequency of replication origins in this DNA. In the case of the bovine satellite I DNA which comprises 7% of the genome, there must be 144,000 copies of the 1,400 base pair repeat. It seems reasonable to assume that the 2,000–3,000 tandem repeats of the 1,400 base pair unit that are present on each autosomal chromosome contain interspersed within them multiple sites for the initiation of DNA synthesis. Alternately, each 1,400 nucleotide repeat may contain such a signal. Reciprocal intrastrand recombination in direct tandem repeats will favour excision and the formation of independent rings. These recombinations, in fact, seem to occur because polymorphism in human centric-heterochromatin indicating deletions in satellite DNA content have been shown to be widespread[31,32]. If a satellite DNA circle formed by such an excision contains a sequence for the initiation of DNA synthesis it may undergo rolling circle replication and amplification. That the enzymes needed for such a process are available in higher cells at meiosis has been shown, as Hourcade *et al.*[33] and Bird *et al.*[34] have demonstrated that ribosomal DNA

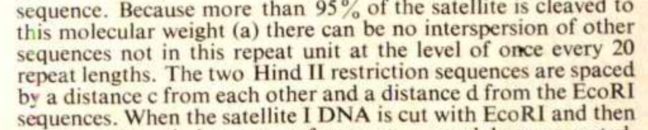

Fig. 5 Restriction map of satellite I DNA. Assuming that this satellite is approximately equally distributed on all 58 autosomes[16] there are 2,000–3,000 repeats of unit (*a*) in each chromosome. This represents the spacing of the EcoRI restriction sequence. Because more than 95% of the satellite is cleaved to this molecular weight (a) there can be no interspersion of other sequences not in this repeat unit at the level of once every 20 repeat lengths. The two Hind II restriction sequences are spaced by a distance c from each other and a distance d from the EcoRI sequences. When the satellite I DNA is cut with EcoRI and then the Hind II restriction enzyme fragments e, c and d are generated. When the satellite DNA is cut with the Hind II enzyme alone, fragments b and c are generated. Partial cleavage products of the double enzyme experiment are: e + c, and c + d. Partial cleavage of Hind II on high molecular weight satellite yields a fragment of size b + c. No polarity to the strand shown can be deduced from the data presented. Therefore, the repeat unit can either be ($^{5'}$–ecdecd–$^{3'}$) or ($^{3'}$–ecdecd–$^{5'}$)●

is amplified by a rolling circle mechanism in *Xenopus* oocytes. The product of this rolling circle replication is a tandem array of identical repeats. To become part of the permanent germ line of the organism, the amplified DNA must subsequently recombine back into a chromosome. This model could account for the origin of new satellite sequences since the repeat that is excised might be sufficiently diverged from the bulk of the satellite DNA. The existence of a previously undetected long range order in satellite DNA is a strong prediction of this model.

The data presented here indicate that two stages were involved in the origin of satellite I DNA in the bovine genome. The first stage involves the small duplications indicated by the renaturation rate to develop the 1,400 base pair repeat, which may have arisen either by a DNA replication process or by unequal crossing over. The second stage involves the production of tandem arrays of this repeat. Should unequal crossing over occur at any point of small homology within the tandem array, regularity of the higher order repeat will be destroyed, because the 1,400 nucleotide units would presumably pair in a staggered orientation. Perhaps the mechanism of recombination could place a restriction upon such staggering (for example, the joining between recombining strands might always include one repeat unit in phase). In view of the contrived nature of this restriction, DNA replication schemes, specifically the rolling circle, seem more feasible for this second process.

Whatever the mechanism involved, amplification may occur rarely. After the satellite DNA has been incorporated into the genome, base changes, insertions and deletions will tend to obscure the homogeneity of the array. In a previous study[35], we have shown that the distribution of EcoRI sites in the mouse satellite is very irregular. We interpreted this to be an indication of this divergence process and that the correction of the repeats in this satellite is either an imperfect or a rare event. But, other restriction enzymes or more complete sequence data on the mouse satellite may indicate a long order organisation to this DNA. It will be important to know whether all satellite DNAs possess a regularity at the level of organisation reported here, or if this is particular to the bovine satellite I.

I thank Dr Joe Sambrook for help in the preparation of this manuscript, Dr J. Maio for advice in isolating the satellite and for use of his Model E centrifuge at various times and Dr P. Sharp for help with the electron microscopy. I have been supported by a Damon Runyon Cancer Research Postdoctoral Fellowship. This work was supported by a grant from the National Cancer Institute.

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letters to nature

Near absence of hard X-ray pulsations in Her X-1

We have previously presented[1] spectral results for Her X-1 in the energy range 20 to 100 keV and have set an upper limit of about 40% for the pulsating component. We have recently made further observations on this source with a telescope of much larger area and have obtained a longer exposure during its bright phase in the binary cycle (phase = 0.25) and almost in the middle of its 'on' period in the 35-d cycle (phase = 0.45 to 0.55). The new observations set an upper limit of 10% for the pulsating component in the energy range 20 to 45 keV which has important consequences on the models proposed for the X-ray emission features of Her X-1.

The balloon flight was carried out from Hyderabad, (17.3° N) on December 12, 1973. The balloon floated at an altitude of 4 mbar and Her X-1 was observed during 0536 to 0654 UT for 4,240 s.

The X-ray telescope, with a field of view 8° × 8°, comprised four NaI crystals each of area 97 cm^2 and thickness 3 mm,

The TAG hydrothermal field

THERE is abundant evidence that seawater penetrates, and circulates within, the oceanic crust at active spreading centres Low heat flow values from ridge crests imply that heat must be removed locally by water circulation[1–6] Furthermore, hydrous metamorphosed oceanic crust and oceanic serpentines, which required voluminous quantities of water during their formation[7–9], also have isotopic compositions which indicate that the isotopic sources had low $\delta^{18}O$ values, such as are found in seawater[10] The tectonic setting of strike–slip and normal faulting along the rift valley scarps of oceanic ridges is conducive to the penetration of dense, cold water down the fractures, the heating of the water at depth, and the exhalation of the less dense hydrothermal water as submarine springs along fracture systems

During the 1972 and 1973 cruises of the Trans-Atlantic Geotraverse (TAG) project of the National Oceanic and Atmospheric Administration (NOAA), extensive bathymetric and geophysical surveys, and dredging, were carried out on an area two degrees square between the Kane and the Atlantis Fracture Zones on the Mid-Atlantic Ridge at 26° N The usual assemblage of pillow basalts, metamorphosed pillow basalts, diabases and gabbros were dredged from scarps within 20 km of the distinct rift valley[11]

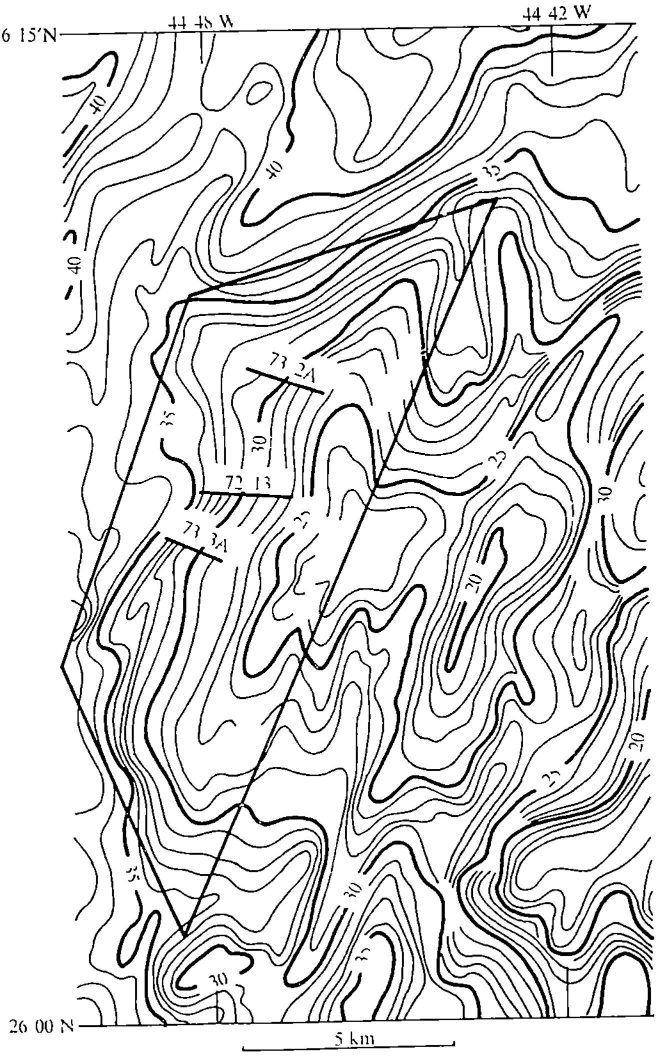

Fig 1 Location of the 1972 and 1973 dredge sites from which hydrothermal manganese was recovered This region covers part of the rift valley in the upper left corner, the eastern wall of the rift valley and a part of the eastern ridge crest highlands[15,35] Contours are in hundreds of metres The TAG hydrothermal field is shown within the solid straight lines which enclose an area of approximately 100 km^2

Along one section of the eastern wall of the rift valley, however, every dredge recovered thick hydrothermal manganese encrustations that overlie and cement underlying talus (Fig 1) Several lines of evidence suggest that this region is an active hydrothermal field

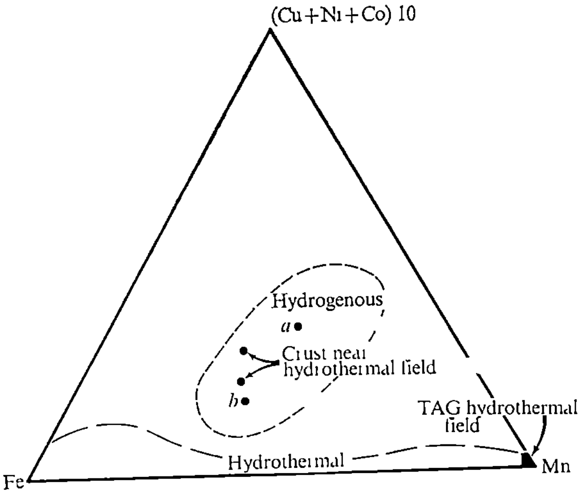

Fig 2 Range of composition of manganese deposits from the TAG hydrothermal field on an Fe–Mn–(Cu+Ni+Co) 10 ternary diagram[16] Average compositions of Pacific (*a*) and Atlantic (*b*) hydrogenous ferromanaganese nodule are from Price and Calvert[34] The two hydrogenous ferromanganese crusts were collected from within 50 km of the hydrothermal field Submarine, hydrothermal ferromanganese deposits have been reported from seamounts, submarine volcanoes, and the Afar and Red Sea spreading centres[16]

The manganese deposit is more than 50 mm thick but is found only 3–8 km from the median axis of the valley and thus must have grown about two orders of magnitude faster than typical open ocean, hydrogenous ferromanganese deposits Rapid growth has been confirmed by $^{230}Th/^{234}U$ and $^{231}Pa/^{235}U$ data which indicate growth rates of 130–250 mm per 10^6 yr respectively[12] The possibility that this rapid growth could be the result of a remobilisation of manganese in reduced, organic-rich, ponded sediment[13,14] is unlikely, because photographs of the ocean bottom[15] snow the hydrothermal deposit growing on essentially bare, current swept talus 500–1,500 m above the rift valley floor Chemically, the deposit is within the hydrothermal region (Fig 2), and mineralogically it consists primarily of birnessite and secondarily of todorokite[12,17]

A positive thermal anomaly (0 14° C) was found in the water column over the hydrothermal region[18] Also, the suspended weak-acid-soluble particles rich in iron and manganese, which were collected over the hydrothermal site, are far more concentrated than similar particles found to either side of the ridge crest[19] Photographs show encrustations covering only the talus slopes[15] Magnetic surveys indicate that the Brunhes normal axial anomaly contains a magnetic low region, the boundaries of which coincide closely with other estimations of the hydrothermal field boundaries[15,35] (Fig 3) Presumably, the hydrothermal alteration of the magnetic mineral component of the pillow lavas has greatly reduced intensity of magnetisation[20] Perhaps most significant, this observation of a distinctive magnetic irregularity associated with hydrothermal activity may become the geophysical exploration tool for the rapid location of new oceanic hydrothermal sites

Ferromanganese deposits and ferromanganese-rich sediments at the interface of basalts and overlying sediments are common features of ophiolites[21–23] Petrological evidence suggests that some ophiolites may have been formed off

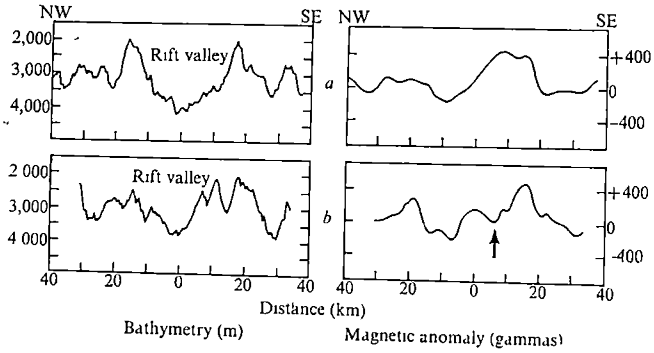

Fig 3 Bathymetric and residual magnetic profiles across the rift valley of the Mid-Atlantic Ridge near 26°N (refs 15 and 35) *a*, 12 km north of the TAG hydrothermal field, showing the strong Brunhes normal over the axial valley, which is typical of all of our profiles from outside the hydrothermal field, *b*, profiles across the TAG hydrothermal field, the arrow indicates the magnetic low in the axial anomaly

ridge axes[24-26], suggesting that hydrothermal mineralisation could occur away from a ridge axis In any case, this does not detract from the probability that the hydrothermal mechanisms that operated in many ophiolitic complexes to form cupreous pyrite bodies[23 27-29], altered pillow lavas, and overlying manganiferous oxides, were similar to that now operating on the Mid-Atlantic Ridge at 26°N Although massive sulphide bodies have not yet been recovered from this site, disseminated pyrite occurs in greenstone pillow lavas dredged from adjacent fault scarps Sulphide deposits are not rare in altered oceanic rocks[30,31] Thus, we infer that the TAG hydrothermal field may at present be forming Troodos-type, massive, stratiform, sulphide bodies within pillow lavas below the manganese deposits

The sharp transition from the precipitation of metal sulphides to the precipitation of metal oxides at the seawater–basalt interface is probably controlled largely by a sharp gradient of oxygen concentration from hydrothermal fluids which have little oxygen, to bottom waters which have relatively high concentrations of oxygen A reasonable estimate of the fugacities of oxygen, and sulphur, and of the *p*H values that may operate in such a system, has been given by Meyer and Hemley[32] The source of sulphur in these hydrothermal systems may in part be reduced sulphate from the influxed seawater, but some sulphur is probably leached from rocks that react with hydrothermal fluids (unpublished work fresh pillow lavas, 1,000 p p m sulphur, adjacent greenstones, 400 p p m sulphur) The iron and manganese are probably leached from crustal rocks under low fO_2 conditions, much of the dissolved iron may be removed from fluids as sulphides at lower temperatures close to the surface, but a significant quantity becomes suspended in the ocean water column[19] Because the iron readily precipitates as hydroxides at lower oxygen concentrations than manganese compounds[33], the particulate iron phase must bypass the manganese crust at the TAG hydrothermal field

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Ancient tropical weathering in Calabria

From the lower Crati valley in Calabria, to the Peloritani Mountains in Sicily, metamorphic and intrusive igneous lithotypes represent the bulk of the Apennine mountain range A wide cover of Tertiary sediment overlies these rocks, mainly along the coastal areas The metamorphic and igneous rocks are in a weathered state The weathering may be tens or even hundreds of metres deep Deep weathering characterises crystalline rocks in a rapidly uplifting region such as Calabria

Current opinion is that the weathering of the intensely fractured crystalline rock masses has resulted from the action of Mediterranean climatic conditions short rainy winters, alternating with long dry summers, and periods of frost[1]

In fact today the area has a meso-Mediterranean type of climate[2] warm dry summers, relatively mild and humid winters and mostly clear, sunny weather throughout the year Rainfall varies from 600 mm yr^{-1} to 1,500 mm yr^{-1}, with about 45% falling during the winter and only 5–8% falling in the summer period, and there is considerable variation from year to year The mean annual temperature is about 17° to 18° C along the coast, about 14° C in the transition zone to the mountains and lower in the highland area[3]

I have collected evidence which suggests that deep weathering is peculiar to tropical rain-forest areas, which have mean annual temperatures between 20° C and 30° C, little annual temperature range and no frost In these areas rainfall may be over 3,000 mm yr^{-1} (ref 4)

In present day and ancient rain-forest tropical areas typical profiles of weathering of crystalline rocks, such as granite or diorite, can be seen Organic sandy soil overlies decomposed rocks of a sandy texture within which the original discontinuities are still preserved (saprolite) The layer is underlain by spheroidally weathered boulders, generally surrounded by sandy material and at the bottom of the profile there are jointed, deeply weathered rocks with sand or clay along the major joints[5]

This is the weathering profile commonly observed in Calabrian granites

Furthermore, there are several conditions, in addition to the presence of a favourable climate, which must be fulfilled before such weathering can occur These include a peneplained landscape, prolonged tectonic quiescence, only a slight amount of erosion, and fracturing to a great depth[6] Only the last of these conditions has been fulfilled in Calabria during the recent geological past, and it is the only condition which is satisfied there today

It thus seems probable that what occurs in Calabria is the intense erosion of an exposed, fossil, deeply weathered mantle This implies that evidence of the necessary conditions will only be found in older geological formations

The deep weathering of Calabrian crystalline rocks is normally considered as an inconsistency which disturbs petrological and structural observations What I have suggested provides the opportunity to understand the tectonic events which occurred since the production of the weathering

Moreover, the need of a better definition of the real relationships between fossil weathered zones and present day morphological features and erosional processes, cannot be disregarded when considering problems of soil erosion and natural slope stability

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Mechanism for producing NO from nitrogen in flames

THE principal chemical reactions which generate the pollutant nitric oxide, NO, from atmospheric nitrogen in combustion systems, are generally recognised[1] to be those first proposed by Zeldovich[2]

$$O + N_2 \rightarrow NO + N \qquad (1)$$

$$N + O_2 \rightarrow NO + O \qquad (2)$$

It is the first reaction in this chain sequence which determines the rate of reaction, and therefore under some combustion conditions reaction (2) may be supplemented by others, such as $N + OH \rightarrow NO + H$ The Zeldovich mechanism is thought[1] to operate generally in flames where oxygen atoms are first produced in the reaction zone, often in concentrations well above those of equilibrium at the temperature which the burnt gases finally attain[3] Measurements[1,4,5] of the rate of production of NO in flames, have revealed a consequent acceleration arising from these superequilibrium concentrations of oxygen atoms

In contrast, Fenimore[6] concluded that NO is produced by an additional mechanism This is thought to operate in or very close to the reaction zone of a flame, and involves a hydrocarbon fragment which attacks molecular nitrogen, for example, $CH + N_2 \rightarrow HCN + N$ Nitric oxide could then be produced by reaction (2), as well as by the oxidation of HCN It should be noted that hydrocarbon radicals such as CH are found generally only in or close to the reaction zone of a flame, because they disappear in the burnt gases downstream by rapid bimolecular processes for example, by $CH + O \rightarrow CO + H$ Because flame gases at atmospheric pressure have a residence time in their reaction zone of no more than 50 µs, the outcome has accordingly been termed 'prompt' NO Although Fenimore's suggestion has been rejected several times[1], particularly in systems which are fuel lean, it has received some support[7,8]

The work reported here represents a preliminary attempt to test whether 'prompt' NO is produced in addition to that from the Zeldovich scheme A fuel-rich flame, of unburnt composition $H_2/O_2/N_2 = 3/1/4\ 75$, was burnt at atmospheric pressure on a water-cooled burner made from stainless steel hypodermic tubes The design of the burner was similar to that described by Padley and Sugden[9], except that it produced only one flame of 16 mm diameter, approximately 0 3 m long, with a flat reaction zone, about 0 1 mm thick, located about 1 mm from the burner face The burnt gases of the flame had a temperature of 2,000 K, which corresponds to an equilibrium concentration for NO of around 7 parts per million (p p m) The gases at various points along the axis of this flame were sampled through a water-cooled probe (made entirely from quartz) into a chemiluminescent NO analyser That instrument gave the concentration of nitric oxide as a function of axial distance along the flame As the radial profiles of velocity, temperature and concentration in the early part of the burnt gases are 'top hat' ones, the system is a good approximation to a one-dimensional flow system Determinations of concentrations, such as [NO], as a function of axial distance, can easily be transformed into functions of time for the first 50 mm of the burnt gases, where the axial velocity is constant, at 11 3 m s^{-1}, and the gases are not disturbed by air entrainment or after-burning Such measurements were made in the flame itself and also with a little of the hydrogen replaced with acetylene to give an overall concentration of 1% by volume of hydrocarbon Such an amount of acetylene in the unburnt gases has been shown[10] not to affect the temperature or composition of the burnt gases, apart from the introduction into them of a small amount of CO Acetylene is, however, likely to release many short-lived radicals into the reaction zone, such as CH and C_2

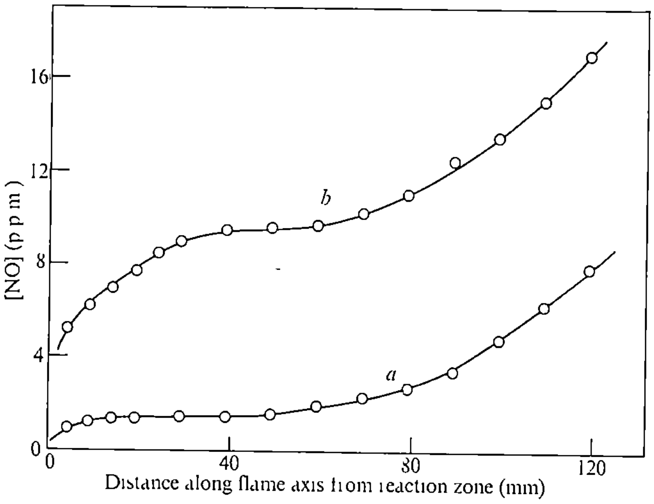

Fig 1 Variation of the concentration of nitric oxide with position along the axis of a flame of unburnt composition $H_2/O_2/N_2$ = 3/1/4 75, and temperature 2,000 K *a*, [NO] without additives to the flame, *b*, burner supplies contain 1% by volume of acetylene

The results are shown in Fig 1 In the absence of added acetylene, [NO] rises sharply in the first 5 mm of the burnt gases and is then almost constant up to about 50 mm (Fig 1*a*) After that, there is another definite increase probably because of the entrainment of O_2 and N_2 from the surrounding atmosphere, and after-burning of the excess hydrogen, with a consequent temperature rise The steady level attained by [NO] around 50 mm downstream, is roughly 0 2 of that expected for equilibrium It is thus clear that the formation of NO, which in this instance must be by the Zeldovich scheme, is slow under these particular conditions, in spite of the fact that oxygen atom concentrations are expected[9,10] to exceed their equilibrium values by up to a factor of 3 × 10^3

In the presence of 1% by volume of acetylene, [NO] rises to a first steady level (at around 40-60 mm) almost seven times higher (Fig 1*b*), which is approximately that for equilibrium Subsequently, [NO] increases downstream because of air entrainment and the gradual temperature increase with after-burning, after the first 40 mm the trends of the two mixtures are parallel (Figs 1*a*, *b*) These observations can be interpreted only in terms of two additive mechanisms for producing NO The Zeldovich scheme is 'singled out' by the first mixture (Fig 1*a*), relating entirely to the simple $H_2 - O_2 - N_2$ system Added to curve *a* is that amount of NO produced in the early part of the flame (although here it does not seem to be confined entirely to the reaction zone but extends to the first 40 mm of the burnt gases) giving curve *b* on the addition of 1% of acetylene This behaviour, which arises from the presence of hydrocarbon, corresponds to the production of 'prompt' NO These data relate to the particular flame conditions used here, and although they demonstrate that, if after-burning is neglected, the 'prompt' NO is almost six times that from the Zeldovich scheme, that is not always so In fact, the ratio of 'prompt' to Zeldovich NO depends on such variables as $[H_2]/[O_2]$ in the unburnt gas supplies, flame temperature, the quantity of acetylene added, and so on These matters will be discussed elsewhere

Table 1 Possible reactions for attacking N_2 in flames

No	Reaction	Standard enthalpy changes at 0 K ΔH J mol^{-1}
(3)	$CH_3+N_2\rightarrow HCN+NH_2$	157±22
(4)	$CH_3+N_2\rightarrow CN+NH_3$	244±12
(5)	$CH_2+N_2\rightarrow HCN+NH$	88±30
(6)	$CH_2+N_2\rightarrow CN+NH_2$	222±30
(7)	$CH+N_2\rightarrow HCN+N$	9±15
(8)	$CH+N_2\rightarrow CN+NH$	200±25
(9)	$C+N_2\rightarrow CN+N$	193±17
(10)	$CO+N_2\rightarrow CN+NO$	636±11
(11)	$HCO+N_2\rightarrow HCN+NO$	240±21
(12)	$HCO+N_2\rightarrow CN+HNO$	560±40
(13)	$H_2CO+N_2\rightarrow HCN+HNO$	350±31
(14)	$H_2CO+N_2\rightarrow HNCO+NH$	337±40
(15)	$CH_3O+N_2\rightarrow HNCO+NH_2$	58±63
(16)	$C_2+N_2\rightarrow CN+CN$	48±25
(17)	$C_2H+N_2\rightarrow HCN+CN$	80±48

Although these observations establish the importance of 'prompt' NO under these conditions, the chemical reaction initially responsible has not been identified We give some of the possibilities in Table 1, together with their associated heats of reaction, for all species in their ground electronic states These enthalpy changes were derived from dissociation energies of diatomic molecules quoted by Gaydon[11] and of polyatomic molecules listed by Herzberg[12], Cottrell[13] or the JANAF Tables[14] Five reactions (5, 7, 15, 16 and 17 in Table 1) seem to be the only candidates for the attack of N_2 by a hydrocarbon fragment, because they have the lowest heats of reaction Admittedly, activation energies can exceed endothermicities, and also the various hydrocarbon radicals do have differing concentrations in a flame But as an increase of 100 KJ mol^{-1} at 2,000 K in the activation energy of a reaction reduces its rate by a factor of 400, we conclude that all the reactions (Table 1), apart from the five mentioned, can be excluded on thermochemical grounds Of the five possibilities, reactions (16) and (17) are unlikely, because they involve a four-centre reaction In the same way, reaction (15) is a very weak candidate, because its transition state lacks plausibility, furthermore, CH_3O is not one of the most common radicals associated with the combustion of a hydrocarbon That leaves reactions (5) and (7) The latter is more likely both sterically and energetically, the primary products of the attack of N_2 are HCN and N If reaction (5) also operates, only one other product, NH, is involved, which is known[15] to have its concentration in these flames linked to that of free nitrogen atoms

$$NH + OH \rightleftharpoons N + H_2O \qquad (18)$$

There is thus very little to distinguish between the products of reactions (5) and (7) In any event, whether NH or N is produced initially, NO is most probably formed[15] by

$$N + OH \rightleftharpoons NO + H \qquad (19)$$

in these hydrogen-rich flames

The other product, HCN, is common to both reactions (5) and (7), as well as to other reactions in Table 1, on account of its stability Its concentration is coupled[16] to that of CN by an equilibration of

$$HCN + H \rightleftharpoons CN + H_2 \qquad (20)$$

with NO resulting[16] from one of two exothermic reactions

$$CN + O_2 \rightarrow CO + NO \qquad (21)$$
$$CN + OH \rightarrow CO + NH \qquad (22)$$

Reaction (22) is followed by reaction (18) and then by reaction (19) In a fuel-rich flame, such as that studied here, $[O_2] \ll [OH]$ and reaction (21) is, therefore, probably unimportant

These considerations indicate that in fuel-rich systems NO can be formed from HCN by a succession of four reactions reactions (20), (22), (18) and (19), whereby $HCN \rightarrow CN \rightarrow NH \rightarrow N \rightarrow NO$ Taking reaction (7), the most probably primary reaction, HCN and N will be produced Both of these will produce NO, but HCN—at the beginning of the sequence of reactions—will do so at a much slower rate than N atoms, which give NO in a rapid, single-step process It could thus well be that roughly half of the NO is generated very quickly, and the rest relatively slowly from HCN, with the result that the appearance of 'prompt' NO could extend into the burnt gases, as observed in Fig 1 In this context, HCN has been observed[7] in the burnt gases of hydrocarbon flames as an intermediate in the production of 'prompt' NO

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Evidence for a complete mixed valence solid solution series in $\mathrm{Fe}_3^{2+}(\mathrm{H_2O})_3[\mathrm{PO_4}]_2$ (phosphoferrite) $\mathrm{Fe}_3^{3+}(\mathrm{OH})_3[\mathrm{PO_4}]_2$ (kryzhanovskite)

HOMONUCLEAR intervalence-transfer absorption of the first transition series cations has aroused considerable interest for two reasons[1] First, it may give rise to intense colour which is not predicted by the addition of the colours of either of the individual ions Second, it may lead in some chain structures to strong anisotropy in electronic conduction A classic example is vivianite, $\mathrm{Fe}_3^{2+}(\mathrm{H_2O})_8[\mathrm{PO_4}]_2$ although colourless when fresh, upon partial oxidation of the transition metal the compound assumes a deep blue which is at its maximum intensity when the electric vector of polarised light is parallel to the axis of the two iron atoms in the octahedral edge-sharing dimer of the crystal structure Extensive study on this and on other systems has led to a considerable body of literature[1]

In the homologous series $\mathrm{Fe}_3^{2+}(\mathrm{H_2O})_n[\mathrm{PO_4}]_2$, where all n water molecules and all oxygens associated with the phosphate tetrahedra are also bound to the inner sphere of the transition metal, extensive oxidation of $n = 8$ (vivianite) and $n = 4$ (ludlamite), leads to destruction of the crystal structure[2] For $n = 3$ (phosphoferrite), it is possible to synthesise the crystalline ferric analogue of a natural mineral with initial composition $(\mathrm{Fe}^{2+}_{2\,1}\mathrm{Mn}^{2+}_{0\,8}\mathrm{Ca}^{2+}_{0\,1})(\mathrm{H_2O})_3[\mathrm{PO_4}]_2$ by heating crystals in air[2] These observations suggest that the balance of electrostatic valence is the basis for the stability of the $n = 3$ homologue over an extensive range of Fe^{2+} oxidation to Fe^{3+}

I have examined single crystals of pure synthetic Fe_3^{2+}-$(\mathrm{H_2O})_3[\mathrm{PO_4}]_2$ with the phosphoferrite structure The use of the pure compound makes it possible to study the complete Fe^{2+}–Fe^{3+} mixed valence series without ambiguities which might arise from other substituents such as Mn^{2+} and Ca^{2+} usually found in natural crystals Crystals of the synthetic compound are pale green with prismatic development parallel to the [100] axis (Fig 1)

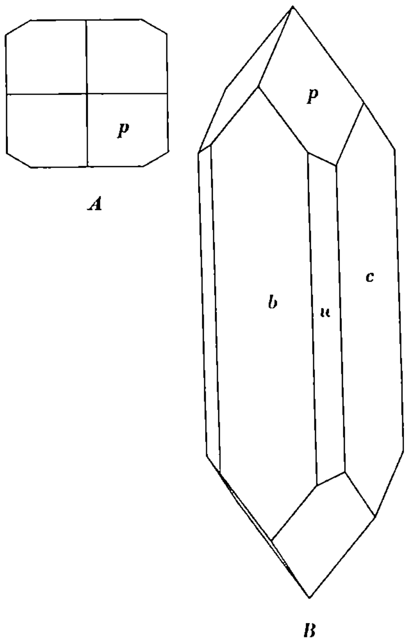

Fig 1 Crystal development of $\mathrm{Fe_3(H_2O)_3[PO_4]_2}$ (phosphoferrite) showing the forms *c*{001}, *b*{010}, *w*{021}, and *p*{111} *A*, Plan, *B*, clinographic projection

Several single crystals were placed on a glass slide and heated in a muffle furnace at 180° C for 24 h The products were deep red and optically transparent with no evidence of decomposition In the early stages of heating, the crystals became progressively darker green Crystals of the ferrous compound were crushed, powdered with Si (a = 5 4301 Å) standard and the mixture uniformly smeared on a glass slide A chart diffractogram with scan speed of 1° min^{-1} in 2θ utilised graphite monochromatised CuKα radiation The same slide was then heated at 180° C for 24 h and another diffractogram was prepared Rotation and Weissenberg photographs were obtained for the ferrous compound and for the same crystal heated at 180° C for 24 h The space group for both crystals was established as *Pbna*

Results of the powder studies, the calculated data obtained by least-squares refinement, are given in Table 1 and the single crystal studies in Table 2 Evidence for the existence of the ferric end member is provided by the deep red colour of the crystal, the volume reduction of about 6 1 Å^3 per Fe atom computed from the cell volume, and from the optical properties of the two compounds The indices of refraction are α 1 678(3), β 1 695(3), γ 1 710(8) with Z || *a*, sign (—), 2V large for the ferrous compound and α 1 873(3), β 1 878(3), γ 1 995(4) with Y || *a*, sign (+), 2V ~ 20° for the ferric equivalent Gladstone–Dale calculations provide densities which are similar to those computed from the unit cell I did not determine specific gravity directly because of the possibility of cracks in the heated compound

The evidence clearly points to a complete solid solution series between $\mathrm{Fe}_3^{2+}(\mathrm{H_2O})_3[\mathrm{PO_4}]_2$ (phosphoferrite) and

Table 1 X-ray powder data for $Fe_3^{2+}(H_2O)_3[PO_4]_2$ and $Fe_3^{3+}(OH)_3[PO_4]_2$

Phosphoferrite $Fe_3^{2+}(H_2O)_3[PO_4]_2$				Kryzhanovskite $Fe_3^{3+}(OH)_3[PO_4]_2$			
I/Io	*d*(obs)	*d*(calc)	*hkl*	*I/Io*	*d*(obs)	*d*(calc)	*hkl*
15	5 379	5 375	111	20	5 189	5 185	111
70	4 997	4 996	020	60	4 867	4 869	020
40	4 722	4 721	200	50	4 751	4 753	200
10	4 320	4 325	021	25	4 269	4 271	210
35	4 272	4 268	210	10	4 010	4 006	022
12	3 931	3 932	121	20	3 812	3 812	121
15	3 431	3 431	220	12	3 399	3 401	220
100	3 190	3 189	221	100	3 128	3 131	221
25	3 087	3 089	122	35	3 064	3 063	202
10	2 958	2 957	301	40	2 942	2 942	122
15	2 757	2 756	103	20	2 679	2 681	230
50	2 721	2 721	230	10	2 591	2 593	222
5	2 687	2 687	222	15	2 573	2 571	103
30	2 656	2 657	113	5	2 523	2 521	321
10	2 495	2 496	023	25	2 489	2 486	113
10	2 465	2 465	312	5	2 436	2 435	040
25	2 413	2 413	123	10	2 407	2 408	312
10	2 327	2 326	141	10	2 342	2 342	023
10	2 218	2 220	411	5	2 329	2 329	041
15	2 212	2 211	331	10	2 271	2 274	123
15	2 160	2 163	042	10	2 262	2 264	213
15	2 108	2 108	142	10	2 220	2 218	411
5	1 9560	1 9556	323	10	2 182	2 182	331
10	1 6109	1 6112	161	10	2 080	2 081	042
20	1.5720	1 5715	352	15	1 9727	1 9733	332
10	1 5449	1 5444	244	15	1 8836	1 8845	422
				10	1 8785	1 8768	341

$Fe_3^{3+}(OH)_3[PO_4]_2$ (kryzhanovskite) In addition, the mosaic spreads of the same reflections for the two compounds were identical within error of the powder diffractometer experiment This suggests that the oxidised equivalent is a single homogeneous crystal One interesting aspect of this solid solution series which involves a ferrous and a ferric end member is that no vacancies need occur over the octahedral sites to maintain charge balance Moore[2] has proposed that the intracrystalline reaction is an auto-oxidation–reduction, that is, $2[Fe^{2+}(H_2O)^0] \rightarrow 2[Fe^{3+}(OH)^-] + H_2\uparrow$

The interesting feature of the phosphoferrite structure includes a kinked octahedral edge-sharing chain which is oriented parallel to the [001] axis of the crystal[2] Equivalent chains are linked to each other only through the corners with Fe-Fe distances considerably longer than the distances within the chains Writing the cell formula $Fe(1)_4Fe(2)_8(H_2O)_{12}[PO_4]_8$ to distinguish the nonequivalent Fe atoms, the sequence of iron atoms in the chain is Fe(1)–Fe(2)–Fe(2)–Fe(1)–Fe(2)–Fe(2)

In the natural type kryzhanovskite with octahedral composition $(Fe^{3+}_{1\ 8}Mn^{2+}_{1\ 0}Ca_{0\ 1}Mg_{0\ 1})$, the ordering scheme is nearly $Fe(1) = Fe^{3+}$ and $Fe(2) = Mn^{2+}_{1\ 0}Fe^{3+}_{0\ 8}Ca_{0\ 1}Mg_{0\ 1}$, according to the structure analysis of Moore[2] It is not known, however, if the Fe(1) site or the Fe(2) site oxidises first or if the oxidation sequence is disordered for intermediate mixed valences of the pure iron compound

Table 2 Crystal cell parameters for $F_3^{2+}(H_2O)_3[PO_4]_2$ and $Fe_3^{3-}(OH)_3[PO_4]_2$

	Phosphoferrite $Fe_3^{2+}(H_2O)_3[PO_4]_2$	Kryzhanovskite $Fe_3^{3+}(OH)_3[PO_4]_2$
a(Å)	9 442(5)	9 505(6)
b(Å)	9 992(4)	9 739(6)
c(Å)	8 644(4)	8 012(5)
V(Å^3)	815 5(1 1)	742 0(1 1)
Z	4	4
ρ (calc)* gm cm^{-3}	3 35	3 66
ρ (calc)† gm cm^{-3}	3 34	3 71

* From the cell volume, formula weight and Avogadro's number

† From the Gladstone–Dale relationship The specific refractive energy for Fe_2O_3 was adopted as $k = 0\ 272$ from Moore[3] for epidotes ($k = 0\ 270$) and from strengite and metastrengite, $Fe^{3+}(H_2O)_2[PO_4]$, ($k = 0\ 274$) The specific refractive energies for the other more accurately known oxides are from Larsen and Berman[4]

Several crucial experiments are in progress including routine refinement of the crystal structures of the ferrous and ferric end members and refinement of the intermediate composition $Fe^{3+}/(Fe^{2+} + Fe^{3+}) = 0\ 33$ This last experiment is particularly important since it should answer the question whether the ordering scheme is $Fe(1) = Fe^{3+}$, $Fe(2) = Fe^{2+}$, $Fe(1) = Fe^{2+}$, $Fe(2) = 1/2Fe^{2+} + 1/2Fe^{3+}$, or $Fe(1) = Fe(2) = 2/3Fe^{2+} + 1/3Fe^{3+}$ In addition, single crystals of a range of compositions can be conveniently oriented for optical study

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Viscoelastic effects in boundary lubrication

THE traditional 'time dependence of static friction' approach, which is used to explain observed variations in static friction, has limited validity[1] The appropriate governing variable is the rate of increase of the tangential force coefficient, $\dot{\theta}$, and not the static contact time as has been suggested previously[1] ($\dot{\theta} = \dot{F}/N$ where N is the normal load forcing opposing surfaces together and $\dot{F}$ is the rate of application of the shearing force)

Experimental work using steel surfaces lubricated by various surface films has shown that surface conditions play a very large role in determining the exact μ_s–$\dot{\theta}$ relationship for a given friction couple where μ_s is the coefficient of static friction The results are briefly summarised in Fig 1 The static friction of steel surfaces, tested when coated with thin oxide films or under 'clean' (high vacuum) surface conditions, was not dependent upon $\dot\theta$ over the given range of $\dot\theta$ values When the surfaces were covered with a monolayer of a metallorganic soap, however, the μ_s–$\dot\theta$ behaviour exhibited the expected dependence on rate The shape of the μ_s–$\dot\theta$ curve (Fig 1) depends on the boundary lubricant involved, but all three curves show an upper and a lower asymptote Exposing the steel surfaces to the laboratory atmosphere results in a similar μ_s–$\dot\theta$ behaviour, presumably because of the adsorption of hydrocarbons on the surfaces This experimental work demonstrates clearly that the static friction of steel can be significantly modified by appropriate boundary lubricants

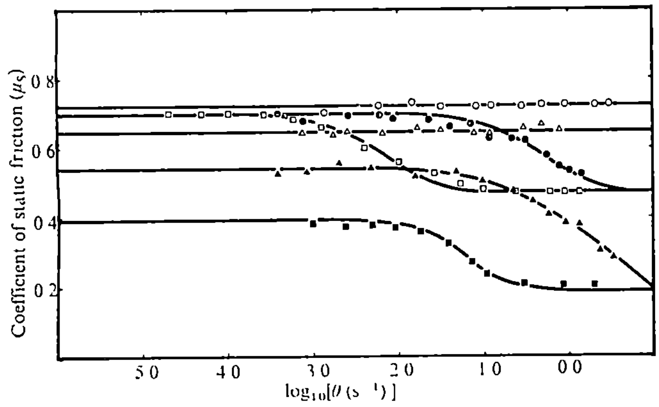

Fig. 1 Static friction, μ_s against $\log_{10}\dot\theta$ for C1020 steel with various surface treatments △, Water-formed oxide, 0 2 × 10^{-8} torr, exposed to $Ca(OH)_2$ in air for 20–48 h—Langmuir Blodgett deposition, □, $Ca(St)_2$—Langmuir Blodgett deposition, ■, $Fe(St)_3$—abrasion technique of Smith and McGill[2]

A model representing the coefficient of static friction, μ_s, as a function of $\dot\theta$ has been developed This model is based on the assumption that the real contact area between the surfaces grows in a viscoelastic manner until a critical shear stress is reached The model has been formulated (see Fig 2) by considering that the interfacial contact region deforms as a three-parameter viscoelastic solid having k_1 and k_2 as its elastic parameters, and c as the viscosity parameter The initial area of contact, A_i, is established by N $A_i = N/p_m$, where p_m is the mean yield pressure of the material Junction growth ends when a critical shear stress, T_0, is reached The μ_s–$\dot\theta$ curve then has the form

$$-\frac{k_1}{p_m}+\left[\frac{k_2}{(k_1+k_2)}\right]\tau\dot\theta\left[1-\exp\left(-\frac{\mu_s}{\dot\theta\tau}\right)\right]+\left[\left(\frac{k_1}{T_0}\right)-1\right]\mu_s=0 \qquad (1)$$

where $\tau = c\left[\frac{(k_1+k_2)}{k_1 k_2}\right]$ is the relaxation time

This equation predicts upper and lower asymptotes for μ_s as follows

$$\mu_{s\,\max}=\frac{T_0}{p_m}\left[\frac{k_1}{(k_1-T_0)}\right] \text{ for } \dot\theta \ll 1 \qquad (2)$$

$$\mu_{s\,\min}=\frac{T_0}{p_m}\left[\frac{(k_1+k_2)}{(k_1+k_2-T_0)}\right] \text{ for } \dot\theta \gg 1 \qquad (3)$$

Using the experimental data and these equations, a relaxation time may be assigned to each of the boundary lubricants For given asymptotes, a larger relaxation time shifts the μ_s–$\dot\theta$ curve to the left This shift may be interpreted as an improvement in the lubrication of the surfaces

In addition, from the experimentally determined relaxation times, a dimensionless μ_s–τ^* curve was prepared for all the boundary lubricants which had the same upper and lower asymptotes All the experimental data fall on to this general curve

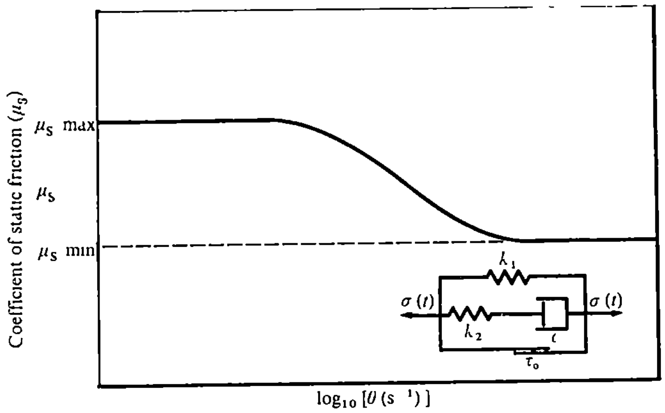
Coefficient of static friction (μ_s)
μ_s max
μ_s
μ_s min
k_1
σ (t)
σ (t)
k_2
c
τ_0
log$_{10}$ [$\dot\theta$ (s^{-1})]

Fig. 2 General form of the μ_s–$\dot\theta$ curve

In a further study, the effect of temperature on the μ_s–$\dot\theta$ behaviour of steel lubricated with a calcium stearate monolayer showed that the relaxation time decreased with increasing temperature These results demonstrated that an increase in temperature produced a progressive deterioration in the quality of lubrication in the context of the shift effect discussed earlier

From equation (1) it is obvious that a 'loading time', where F is constantly increasing, and a 'delay time', where F is held constant, are not equivalent in their effect on area growth For the calcium stearate monolayer case, for example, it was calculated that for a delay time of 1 s, the contact area is increased by 0 5% Even if the delay time is infinite, the area increase is only about 10% These calculations reveal why delay times will not increase significantly μ_s values, and why measurements of the interfacial electrical resistance[1] do not indicate appreciable area growth during the delay period

Thus, the transverse load rate dependence of static friction may be associated with the viscoelastic properties of lubricant monolayers It is believed that the viscoelastic explanation of the observed friction behaviour reflects more accurately the physical processes involved in the sliding of one body over another

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An auditory illusion

I HERE report a novel and striking auditory illusion, which provides a paradox for theories of pitch perception and auditory localisation[1,2], and which varies in correlation with the handedness of the listener The stimulus configuration which produced the illusion consisted of a sequence of tones, alternating in pitch between 400 Hz and 800 Hz (Fig 1*a*) Each tone lasted 250 ms, with no gap between tones The sequence was presented at equal amplitude to both ears simultaneously; however, when one ear received 400 Hz the other received 800 Hz, and *vice versa* Thus the same two-tone combination was presented constantly, but the ear of input for each component switched every 250 ms

It is easy to imagine how this pattern should sound if perceived correctly However, 86 subjects listened to a 20-s presentation of this sequence, and none obtained the correct percept Instead, various illusory percepts were reported (Table 1), the most common being a single tone oscillating

Table 1 Categories of illusory percept

	Octave (%)	Single pitch (%)	Complex (%)
Right handers ($n = 53$)	58	25	17
Left handers ($n = 33$)	52	9	39

Figures show the percentages of right or left-handed subjects obtaining a given percept The two groups of subjects differed significantly in the relative distribution of their percepts (χ^2 = 6 8, d f = 2, P<0 05) Octave indicates the illusion of a single tone oscillating from ear to ear, whose pitch oscillates synchronously from one octave to another Single pitch indicates the illusion of a single tone oscillating from ear to ear, whose pitch either remains constant or shifts very slightly Complex comprises various complex percepts, such as two alternating pitches in one ear, with a third pitch intermittently in the other, or two alternating pitches oscillating from ear to ear and two further pitches alternating at twice the speed localised at the back of the head This group of percepts tends to be quite idiosyncratic and unstable, however, no subject reported the stimulus correctly

from ear to ear, whose pitch also oscillated from one octave to the other in synchrony with the localisation shift (Fig 1*b*) Two subjects with absolute pitch identified these oscillating pitches as G_4 (392 Hz) and G_5 (784 Hz), these are closest on the musical scale to the 400 Hz and 800 Hz presented The percept depicted by one of these subjects is shown on Fig 2

This synchronous alternation of apparent pitch and localisation is paradoxical The perception of alternating pitches can be explained by assuming that the listener processes the input to one ear and ignores the other But then both the alternating pitches should seem to be localised in the same ear Alternatively, the oscillation of a single tone from ear to ear can be explained by supposing that the listener suppresses the input to each ear in turn But then the pitch of this tone should not change with a change in its apparent localisation

A further surprise involves the patterns of apparent localisation for the two pitches at the two ears in right and left-handed subjects Right handers had a highly significant tendency to hear the high tone in the right ear and the low tone in the left (P < 0 001, two-tailed on a binomial test), and also to maintain a given localisation pattern when the placement of the earphones was reversed (P < 0 001, two-tailed on a binomial test) Left handers did not localise the tones preferentially either way, however, though they showed a marginally significant tendency to retain a given localisation pattern when the earphones were reversed (P - 0 05, two-tailed on a binomial test) This difference between right and left handers is consistent with findings relating patterns of hemispheric dominance to handedness[3,4] and so suggests that we tend to localise the high tone to the side producing the most effective input to the dominant hemisphere[5-7] and the low tone to the other side

The localisation patterns on Table 2 are based on the two initial presentations of the sequence, however, with continued listening these patterns sometimes reverse, as in reversals of ambiguous figures in vision[8] Thus these localisation patterns represent strong tendencies, rather than absolute rules

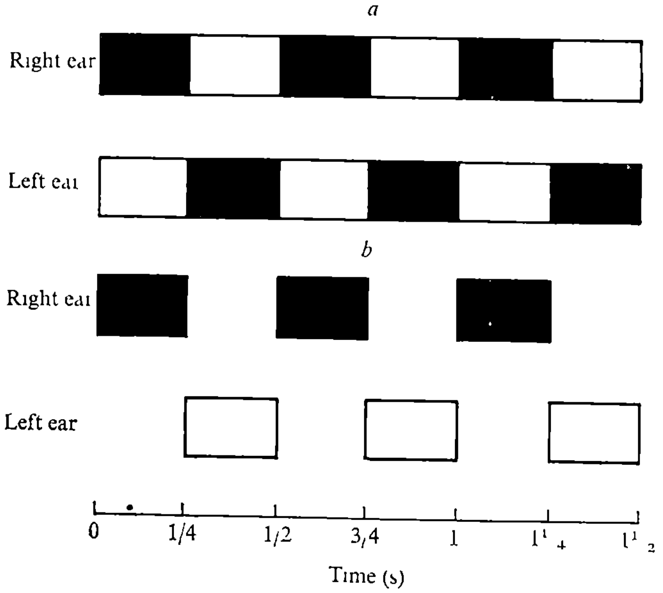

Fig 1 *a*, Representation of the stimulus configuration which produces the illusion Filled boxes represent tones of 800 Hz and unfilled boxes tones to 400 Hz The tones were sinusoids, and their phase relationship varied randomly They were played to subjects at a level of 75 dB SPL *b*, Representation of the illusory percept most commonly obtained

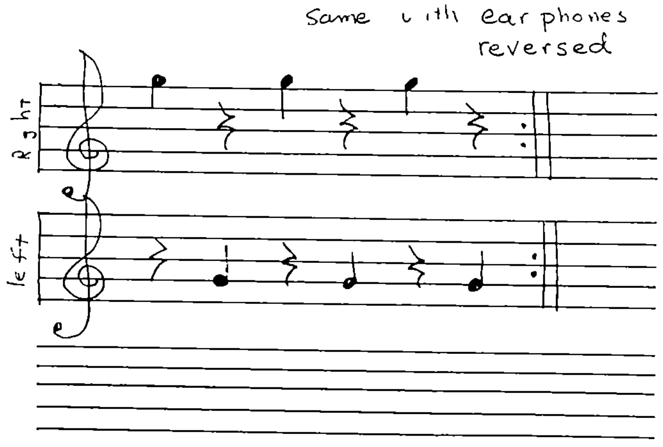

Fig 2 Percept of the stimulus depicted by a subject with absolute pitch The writing is that of the subject herself Her statement 'Same with earphones reversed' shows that the high tones were localised in the right ear and the low tones in the left, irrespective of the positioning of the earphones

Table 2 Patterns of apparent localisation for the two pitches at the two ears in subjects who obtained the octave illusion

	RR	LL	Both
Right handers	25	5	1
Left handers	6	7	4

Each subject was twice given a 20-s presentation of the stimulus sequence, with earphones placed one way initially and then reversed The order of earphone placement was strictly counterbalanced for both right and left-handed subjects Figures show the number of right or left-handed subjects obtaining a given localisation pattern RR, high tone localised in the right ear and low tone in the left on both stimulus presentations LL, high tone localised in the left ear and low tone in the right on both stimulus presentations Both, high tone localised in the right ear and low tone in the left on one stimulus presentation, and high tone localised in the left ear and low tone in the right on the other

The question arises whether a given localisation pattern depends on absolute or relative pitch levels in the stimulus configuration To determine this, I selected twelve right handers who had consistently localised the 800 Hz tone in the right ear, and the 400 Hz tone in the left, showing no tendency to reverse this pattern over several listening sessions These subjects were presented with tape segments consisting of tones alternating between 400 Hz and 800 Hz, between 200 Hz and 400 Hz, and between 800 Hz and 1,600 Hz, the other parameters being identical These sequences were presented for 20 s each in counterbalanced order, with earphone positions also counterbalanced Except for one subject s report on the 200 Hz–400 Hz sequence, the higher of the two tones in each pattern was always localised in the right ear and the lower in the left I conclude that these localisation patterns depend on the pitch relationships between the competing tones, and not on a pattern of ear preference at different pitch levels Further, when I presented the two sequences channelled through spatially separated loudspeakers instead of earphones, the illusion was still obtained, even though both sequences were now presented to both ears, with only localisation cues to distinguish them.

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Maternal behaviour and the effects of stress in tree shrews

THE tree-shrews (family Tupaiidae), small squirrel-like mammals occurring throughout South-east Asia, are currently classified[1] into two subfamilies: Ptilocercinae (nocturnal pen-tailed tree-shrews; genus *Ptilocercus*) and Tupaiinae (diurnal tree-shrews; genera *Tupaia, Anathana, Dendrogale, Lyongale, Urogale*). The overall systematic status of the family is still controversial. The tree-shrews are variously allocated to the Primates[2–3], Insectivora or to an entirely separate order of mammals[4–6]. Recently the unusual maternal behaviour of one tree-shrew species, *Tupaia belangeri*, together with other reproductive characteristics, has been interpreted as further evidence excluding tree-shrews from the Primates[1]. Key features of maternal behaviour in captivity are that the mother deposits her young in a separate nest subsequently visited for suckling only once every 48 h. If such behaviour and its functional correlates are typical of all tree-shrew species, arguments based on reproductive peculiarities could be more confidently integrated with considerations of methodology, general morphological evidence[7], detailed information on the central nervous system[8] and the results of biochemical investigations[9] to reach a balanced assessment of the phylogenetic relationships of tree-shrews.

A comparative study has now been carried out by one of us (F.D'S.) on the reproduction of three tree-shrew species representative of the adaptive range within the subfamily Tupaiinae (possibly excluding *Dendrogale*). The study included one of the largest, predominantly terrestrial species *Lyonogale tana* (average body weight 200 g), the intermediate, semiarboreal *Tupaia belangeri* (*cf. T. glis* average body weight 145 g), and the smallest, extensively arboreal species *Tupaia minor* (average body weight 44 g)[10]. Details of laboratory maintenance and observation are provided by Martin[1]. In the period May 1971 to February 1973, twenty litters were born (a total of forty-three infants: sixteen *T. minor*; sixteen *T. belangeri*; eleven *L. tana*). Thirty (70%) of the infants survived to sexual maturity (thirteen *T. minor*; ten *T. belangeri*; seven *L. tana*). Data have also been collected on three additional litters (six infants) of *T. belangeri* reared by their mothers in Martin's laboratory.

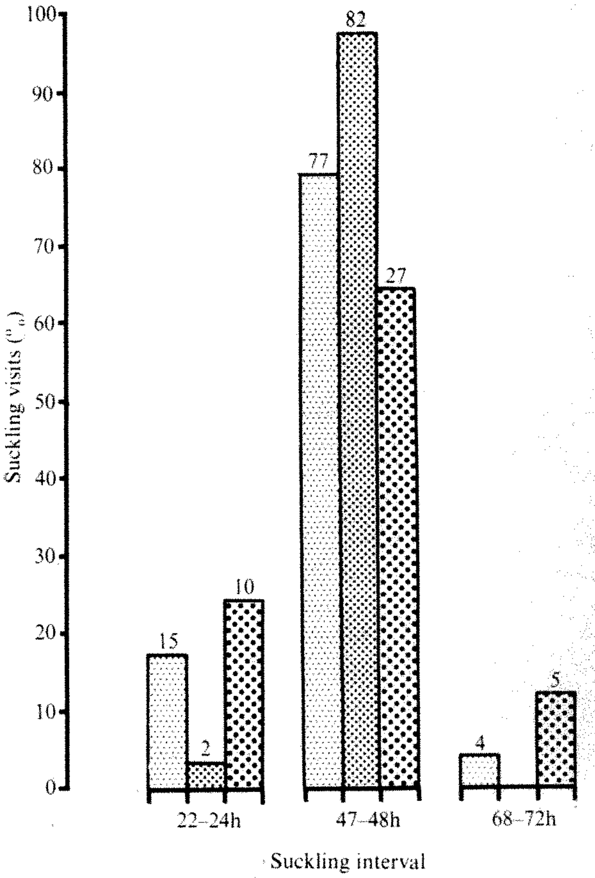

Fig. 1 Histogram of suckling intervals for the three tree-shrew species. Fine stippling: *T. minor*; Medium stippling: *T. belangeri*; Coarse stippling: *L. tana*. The three limited ranges shown on the abscissa cover all the observed suckling intervals. Numbers above the bars indicate numbers of observations.

One of us (F.D'S.) has now demonstrated, under established conditions[1], that both *T. minor* and *L. tana* also typically exhibit 48-h suckling intervals whilst rearing their young in separate nest-boxes (Fig. 1). Accordingly, weight curves (Fig. 2) show a characteristic zig-zag pattern, since the milk continues to be digested without replenishment over each 48-h period. *L. tana* and *T. minor* infants, like *T. belangeri*, maintain a constant body temperature of about 36° C from birth onwards. With all three tree-shrew species, the amount of milk given to the litter by the mother at each suckling visit is considerable (*L. tana* average, 9.4 g; median, 9.4 g; *T. belangeri* average, 4.8 g; median, 5.0 g; *T. minor* average, 1.8 g; median, 2.0 g). Analysis of the milk of the three species shows that both the calorific density and the fat calorie content are extremely high (total solids: *L. tana*, 33.5%, *T. belangeri*, 40.4%, *T. minor*, 44.6%. In all three species: fat, >17%; protein, 6–11%; lactose, <2.5%.) It has been suggested[1] that this milk composition is necessary for constant maintenance of an elevated body temperature by the young. In addition, the solid content of the milk is inversely proportional to the adult and infant body size of the species concerned. The high protein content of the milk is consistent with the normal rapid growth rate of the young (Fig. 2). In all three species, as reported for *T. belangeri*[1], each suckling visit made by the mother is very short. The quantity of milk (q) given increases with the species' body size, but the duration (d) of the suckling visit decreases with increasing body size, means for 3 litters each: *L. tana* q=9.4 g (2.0–19 g) (1.0–11 m); d=3.9 min; for *T. belangeri*, q=5.0 g (1.0–10 g); d=5.8 min (2.5–9.5 m); for *T. minor*, q=2.0 g (1.0–6.5 g); d=6.6 min (2.5–10 m). The small litter sizes of the three species (usually one to three) are probably constrained by the minimum infant body weight consistent with maintenance of a constant elevated body temperature (compare Fig. 2) and by the upper limit of milk production by mother tree-shrews. In fact, one unusual litter of four infants was successfully reared by a *T. belangeri* mother; but the amount of milk given to the litter as a whole was little different from that given to typical litters of two or three young (average total amount of milk given during nest phase to three litters of two is 153.6 g (range 134–172 g); total given to litter of four was 181.0 g).

Thus this peculiar pattern of maternal behaviour is, under appropriate conditions, typical of *T. minor*, *T. belangeri* and *L. tana*, with only minor variations according to body size. Circumstantial evidence indicates that *Urogale everetti*[11] behaves similarly and, although no evidence is yet available for *Dendrogale* it seems that this 'absentee system' of maternal care might be typical for the Tupaiinae. It remains to be seen whether *Ptilocercus* possesses the same characteristics. Certainly, it is difficult to reconcile the pattern found in the Tupaiinae with the suggestion that tree-shrews are derived from the ancestral primate stock, since all living primates are characterised by the advanced condition of the young at birth and by the great elaboration of mother–infant contacts[12].

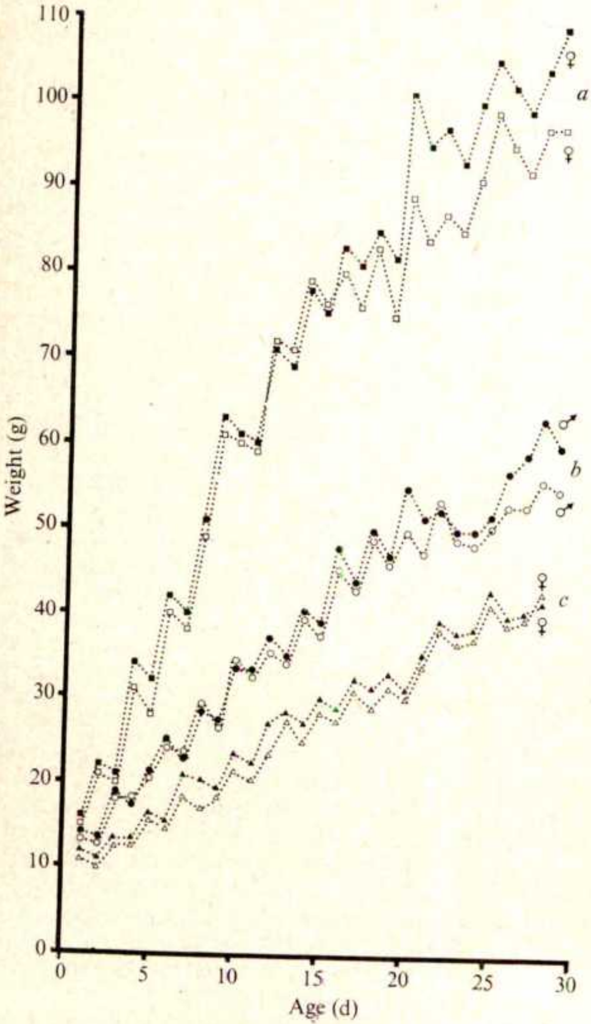

Fig. 2 Weight curves for single litters of two young per litter, for *L. tana* (*a*); *T. belangeri* (*b*); *T. minor* (*c*). The birth weights of the three species are relatively similar but weights diverge considerably by the end of the nest phase. The dip in *T. belangeri* (*b*) weights at 3 weeks coincided with the arrival of a new laboratory technician (an event known to disrupt tree-shrew maternal behaviour[1]).

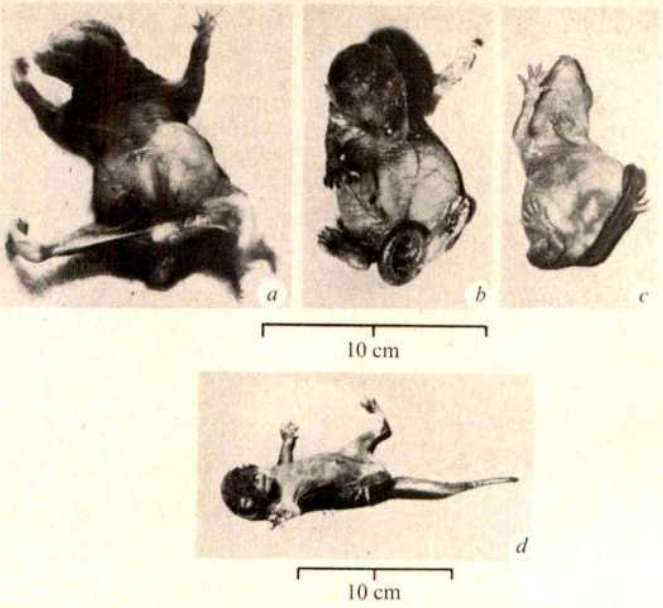

Fig. 3 Neonate *L. tana* (*a*); *T. belangeri* (*b*); *T. minor* (*c*), showing stomachs distended with milk ingested immediately after birth. At the end of each 48-h interval between suckling visits the stomach is deflated. (*d:* 10-d-old *T. belangeri* just before suckling). Note the altricial condition of the neonates.

Sorenson[13] has recently questioned the occurrence of this pattern of maternal behaviour in tree-shrews and has suggested that the 48-h rhythm may be peculiar to *T. belangeri*. Earlier careful studies, however, did not disclose such behaviour in this species and it is surprising that Sorenson did not observe similar behaviour with *Tupaia longipes*, a very close relative of *T. belangeri*. D'Souza's subsequent observations of the "absentee system" in both *T. minor* and *L. tana* (Fig. 3) conflict with Sorenson's account and a laboratory artefact may have been involved at some point. A clue is provided by von Holst's[14] observations that mild social or general environmental stress will distort maternal behaviour in *T. belangeri*, so that the suckling intervals become shorter but less milk is given over a 48-h period. (More severe stress leads to cannibalism of infants and eventually to sterility.) An example of such mild disruption was produced in Martin's laboratory by two fire-bell tests at a time when a female *T. belangeri* was rearing infants. After each fire alarm, the suckling rhythm accelerated, but the quantity of milk given over the 48 h was reduced by about 30% below expectation. The outcome of the two bell tests (Fig. 4) was that the development of the young was retarded (for example the eyes opened 3 d late and emergence from the nest was delayed by 4 d). Thus where the 48-h suckling rhythm has not been observed with tree-shrews breeding in captivity, the composition of cage groups and/or the general environmental conditions provided may inadvertently have led to modification of maternal behaviour. This interpretation is entirely supported by an examination of infant mortality rates. Martin achieved infant survival rates of about 80% with pairs of *T. belangeri*, kept under improved conditions[1], whereas D'Souza has attained survival rates of 70% with all three species. This compares with survival rates of 25% on average (mortality rates of 75%) with various colonies in which the 48-h rhythm was not apparent. An exception occurred with a colony maintained by Kuhn and Starck[15], where females were kept isolated after mating and where infant survival rates of 60% were achieved; but no data on maternal suckling behaviour were provided. In fact, Conaway and Sorenson[16] hand-reared many of their tree-shrew offspring because infant mortality was extremely high. In a study conducted by Sorenson and Conaway with *Tupaia montana*[17], the mortality rate of the offspring born in captivity was apparently 100%. If the survival rate can be taken as a criterion of the degree of immunity of maternal behaviour from disruption in captivity, it seems likely that the 48-h suckling rhythm is the more 'natural' pattern, although no relevant field observations have yet been conducted.

As one further test of the 48-h suckling rhythm in *T. belangeri*, Martin removed infant tree-shrews five times from the nest for periods of about 36 h between suckling visits to see if this had any effect on maternal behaviour. The weight curves of the infants ranked in the upper range recorded for the species[1]. It is striking that total removal of the infants from the nest for long periods has virtually no effect on tree-shrew maternal behaviour, whereas two 30-s fire-bell tests can produce marked disruption. The results reported here should establish that the 48-h suckling rhythm is indeed typical of several members of the Tupaiinae when maintained under relatively stress-free conditions, whilst at the same time providing a cautionary tale for research workers concerned with the interpretation of behaviour in captive colonies of animals.

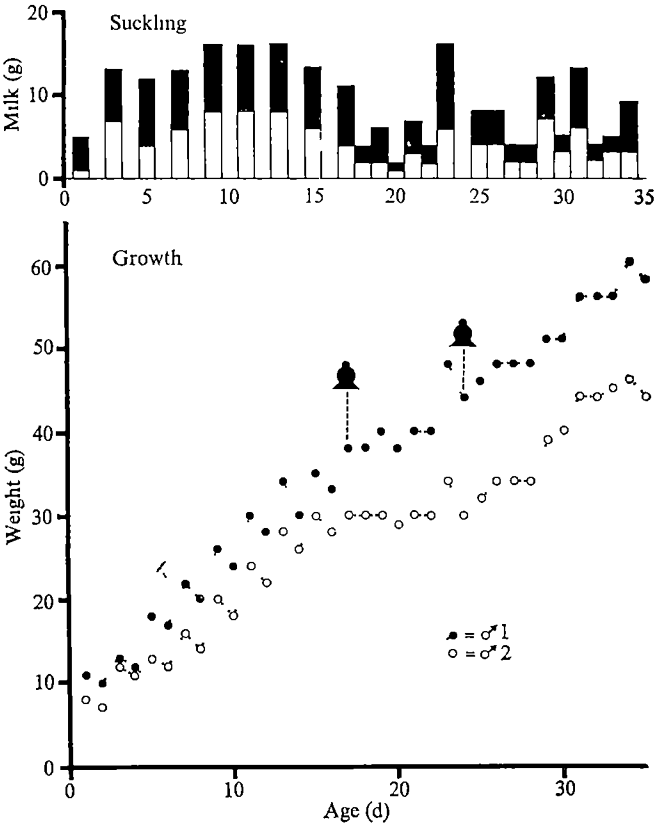

Fig. 4 Weight curves (lower) and histogram showing milk intake (upper) for a litter of *T. belangeri*. Two fire-bell tests were carried out on days 16 and 23, in both cases resulting in disruption of the 48-h suckling rhythm, reduction in milk intake per 24 h and depression of the weight curve. ■, ♂ 1, □, ♂2.

The analysis of tree-shrew milk was carried out by R. L. J. Lyster, National Institute for Research in Dairying, Herr K. Noack, Allgauer Alpenmilch Gesellschaft (after Martin[1]), and Dr D. L. Frape, Spillers Ltd. We thank Dr Gilbert Manley for a critical reading of the manuscript.

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Trace element content and body size in molluscs

WHEN trace element concentrations in shellfish are expressed on a weight-specific basis (as $\mu g\ g^{-1}$) highest values are often recorded in the smallest individuals[1]. In these cases, it is difficult to assess whether observed differences in element tissue concentrations between populations reflect real differences in environmental trace element constitution, or are merely due to variations in body size. This problem can be avoided by determining element concentrations over a range of body sizes and reference made to a specific size for comparative purposes.

Element concentrations have been measured in individual molluscs over a wide size range. Six estuarine species have been examined: limpets, *Patella vulgata* L. and *P. intermedia* Jeffreys; the cockle, *Cerastoderma edule* (L.), the mussel, *Mytilus edulis* L.; and clams, *Mercenaria mercenaria* L. and *Venerupis decussata* (L.). Limpets were collected from Portishead, on the Severn Estuary and the River Helford, Cornwall, cockles from Southend, Essex, *M. mercenaria* from Southampton Water, and *V. decussata* and *M. edulis* from Poole Harbour, Dorset.

Nitric acid digestion techniques were employed, trace element concentrations being determined by atomic absorption spectrophotometry in a final volume of 5–20 cm^3 1 M HCl (with additional dilution if necessary). Theoretical instrument sensitivities for the various elements in the minimum volume used of 5 ml are Cd 0.05, Cu 0.1, Fe 0.5, Ni 0.5, Pb 0.5 and Zn 0.05 μg. Because such limits apply only to pure solutions, not subject to matrix effects found in digestates of animal tissues, minimum amounts considered were generally at least five to ten times greater than the above values. Concentrations obtained have been expressed as the content (μg per individual) and as the weight-specific concentration ($\mu g\ g^{-1}$). Both have been plotted against dry body weight (g). Regression lines were fitted by the method of least squares and all were significantly correlated at the 0.1% level.

The method of statistical analysis employed is analogous to that relating functions of metabolism to body weight[2,3]. Total trace element content per individual Y relates to body weight W as a power function expressed by:

$$Y = aW^b \qquad (1)$$

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which on logarithmic transformation yields a regression of the form

$$\log_{10} Y = \log_{10} a + b \log_{10} W \qquad (2)$$

The weight-specific relationship Y′ to body weight is essentially similar. Dividing (1) throughout by W yields

$$Y' = Y/W = aW^{b}/W = aW^{b-1} \qquad (3)$$

Logarithmic transformation produces a linear function

$$\log_{10} Y' = \log_{10} a + (b-1) \log_{10} W \qquad (4)$$

Clearly equations (2) and (4) have related regression coefficients and share a common intercept (Y and $Y' = \log_{10} a$ when $W = 1.0$, $\log_{10} W = 0$).

The precise relationship between trace element content (μg) and dry body weight (g) differs depending upon element and species. Three general relationships have been identified (Figs 1–4 and Table 1). First, element content is related to $\simeq$ 0.75 power of body weight. This relationship was true for copper in all species examined. A variety of other elements exhibited the same relationship (Table 1), the actual values obtained for the regression coefficients of 32 lines (relating to the various elements in the six species examined) ranged from 0.67 to 0.85. Covariance analysis yielded no significant difference between pairs of coefficients. Pooling the sums of the squares and sums of the products of deviations from the regression[4] gave a common regression coefficient of $b = 0.77 \pm 0.04$ (weight-specific slope -0.23) (Figs 1 and 2).

Second, element content is directly related to body weight ($b \simeq 1.00$). Nickel in all bivalve species examined and also lead and zinc in *M. mercenaria*, iron and zinc in *V. decussata* and cadmium in *M. edulis* exhibited this relationship to body weight. The actual regression coefficients of 15 lines varied from 0.97 to 1.17, no pair of slopes being significantly different from one another. These lines yielded a common regression coefficient of 1.03 ± 0.02. In these cases tissue element concentrations are independent of size (Fig. 3).

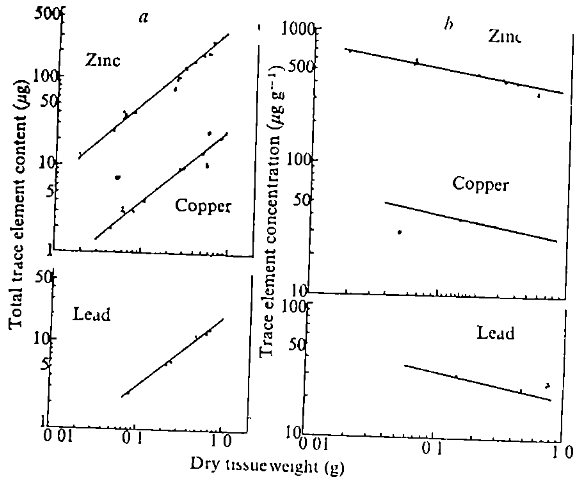

Fig. 1 Relationship of *a*, element content (μg) and, *b*, element concentration (μg g⁻¹) to body weight (g) for copper, lead and zinc in the limpet *Patella vulgata* collected on August 3, 1973 from Portishead, Severn Estuary.

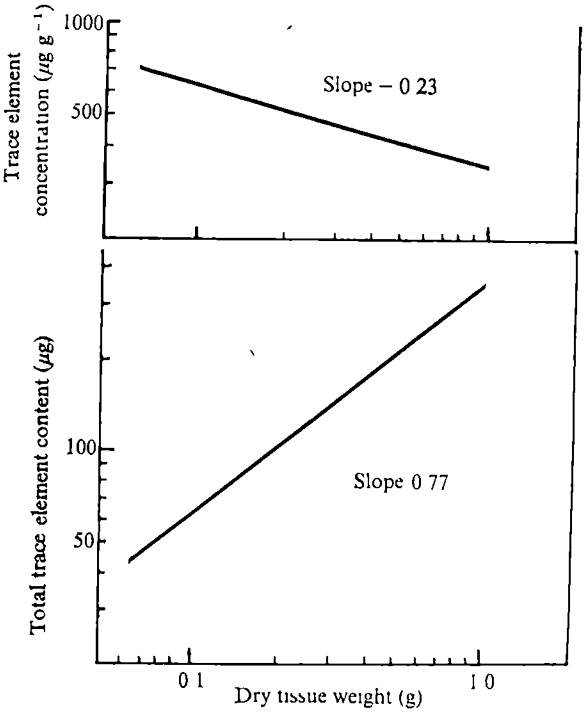

Fig. 2 Relationship of element content (and concentration) to body weight where element content related to 0.77 power of body weight. The data shown pertains to zinc in *P. vulgata* collected from Portishead, Severn Estuary.

Third, element content is related to square of body weight ($b \simeq 2.00$). This pattern was only observed for a single element, cadmium, in the limpet *P. vulgata* (Fig. 4). Therefore in this specific case, element tissue concentration is directly proportional to body weight.

Some earlier observations in the literature are consistent with the above relationships. A regression slope of 0.77 (weight-specific slope of -0.23) dictates that smaller individuals contain a higher concentration of metal than larger individuals. Such differences have been recognised for lead in *M. edulis*[1], zinc in *P. vulgata*[5] and copper and iron in *M. mercenaria*[6]. The converse situation, with higher concentrations of cadmium in large limpets compared with small individuals, has previously been reported for *P. vulgata* collected from the Severn Estuary[5,7]. In the molluscs studied, however, it was usual for element concentrations to decrease or remain constant with increasing body weight. Species from other phyla would be expected to exhibit similar relationships, certainly concentrations of

Table 1 Distribution of the three types of regression coefficients relating element content (μg) to dry body weight (g) within six species of mollusc

Regression coefficient	Cadmium	Copper	Iron	Nickel	Lead	Zinc
$\simeq$ 0.75	*C. edule* *M. mercenaria* *V. decussata*	*P. vulgata* *P. intermedia* *C. edule* *M. edulis* *M. mercenaria* *V. decussata*	*P. vulgata* *P. intermedia* *M. edulis* *M. mercenaria*		*P. vulgata* *P. intermedia* *C. edule* *M. edulis* *V. decussata*	*P. vulgata* *P. intermedia* *C. edule* *M. edulis*
$\simeq$ 1.00	*M. edulis*		*C. edule* *V. decussata*	*M. edulis* *C. edule* *V. decussata* *M. mercenaria*	*M. mercenaria*	*V. decussata* *M. mercenaria*
$\simeq$ 2.00	*P. vulgata*					

cadmium, copper, iron, manganese and zinc either decrease, or remain constant, in whole fish when expressed as a function of size, weight or length[8]

In molluscs, as in biota in general, absolute tissue element concentrations are related or determined by environmental concentrations Within each species, however, different elements display different relationships to body weight (Table 1) Regression coefficients related to body weight by a power of 0 77 implies a connection with metabolism, as many metabolic processes display a similar relation to body weight The common regression coefficient of 0 77 ± 0 04 is not significantly different from the coefficient of 0 75 ± 0 015 describing the relationship of respiration of poikilotherms to body weight[9], a value generally accepted to relate many metabolic functions to body weight Thus in cases where an element is related to 0 77 body weight some aspect of metabolism could well be influencing final trace element content

Where the element content is directly related to body weight, as has been found for a variety of elements in several species, then a function of body weight such as the binding of specific compounds within the tissues may play some role in determining the total body element burden The absolute amount of metal, however, is not dictated by the amount of such binding compounds within the tissues At least certain metals such as cadmium and nickel in *Mytilus*, retain a specific slope relation of ≃ 1 00 between total body element content and body weight, even though the absolute amount of the metals within the tissues differed by twelvefold for cadmium and twofold for nickel between two separate populations studied

A relationship to the square of body weight as shown by cadmium in limpets can best be explained as being due to removal of this element from body circulation and accumulation within specific tissues, possibly as a result of some exceptional affinity The slope value of 2 00 in the limpet population examined, may well be due to the unusually elevated environmental cadmium concentration in the Severn Estuary Populations of limpets from other areas, where environmental cadmium concentrations are lower and more normal, exhibit slopes between 1 3 and 1 5 which may well indicate that in this specific case, the slope is variable and related to the environmental cadmium concentration

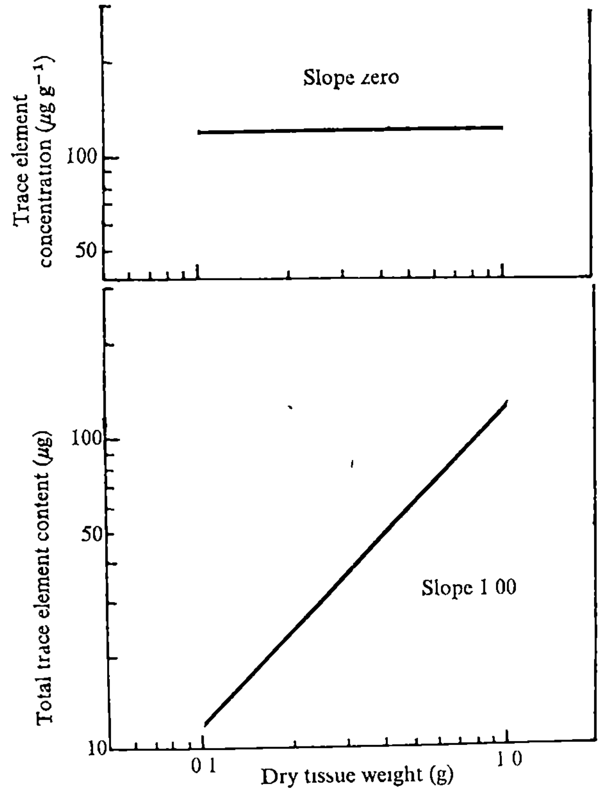

Fig 3 Element content directly related to body size, weight-specific concentration independent of size This example relates to nickel in the cockle *C edule*

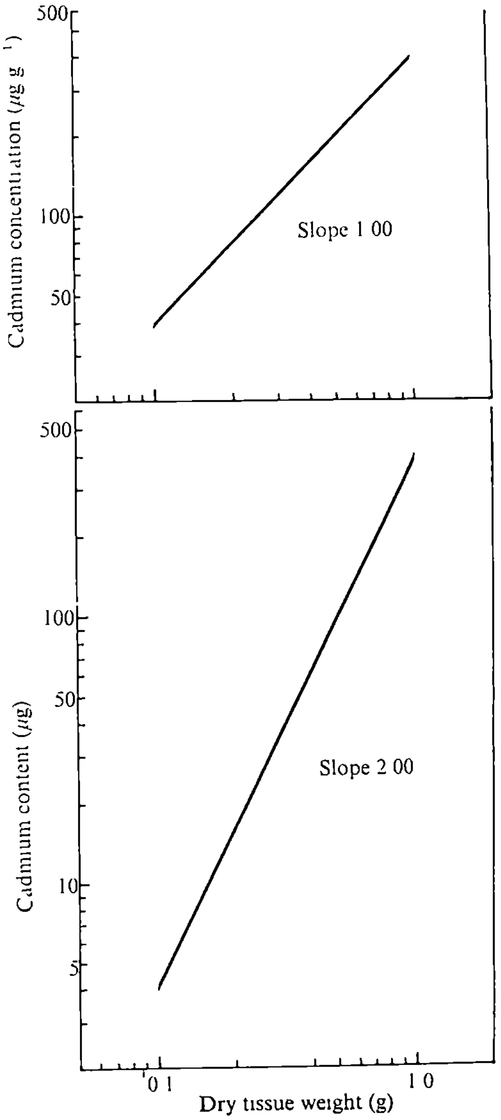

Fig 4 Relationship of cadmium content (and concentration) to body weight in the limpet *P vulgata* where cadmium tissue concentration (μg g^{-1}) is directly related to body weight Limpets collected from Portishead

Knowledge of the regression coefficients of lines relating trace element content (or the weight-specific concentration) to body weight is potentially a considerable aid in the monitoring of metals within shellfish Evidence is being gathered which suggests that in most cases the regression coefficient relating element content to body weight remains constant irrespective of season or environmental element concentration The only case so far identified where the slope differs between populations is that for cadmium in *P vulgata* discussed above, though other exceptions may also be expected If values of *b* are indeed constant for a particular element within a species, data pertaining to element content or concentration and mean tissue weight for a sample of similar sized individuals may be substituted, together with the appropriate slope for the element and species in question, into equations (2) or (4) The calculated intercept ($\log_{10}a$) converted to real values may then be used for comparison between populations irrespective of the size of the individuals sampled and analysed The influence of seasonal variations and environmental element concentrations on the above relationships is receiving further investigation

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LSD treatment of *Pieris brassicae* and consequences on the progeny

At 20° C, under white light illumination and circadian rhythm, nymphal diapause of *Pieris brassicae* is dependent on a short 9-h photophase Some psychodysleptics (mescaline sulphate and LSD), when injected during the photosentitive larval period, suppress diapause induction as if the larvae were subjected to a long 16-h photophase[1] Abnormalities in the behaviour of the progeny of treated animals were observed We studied them for three generations after the treatment

Three couples of *P brassicae*, progeny of a single original pair, were isolated The progeny of each of these pairs were divided into two groups, one served as control (normal stock) and the other was treated with LSD Control and treated animals of each progeny, respectively, were pooled at the end of each experiment after assessment of the fluctuation Both stocks were subjected to a 9-h white light photophase treatment at 20° C (14,600 erg cm^{-2} s^{-1} with a Mazda TFR/40/BBL lamp)

LSD was diluted in Ringer solution to different concentrations Animals were injected with 5 μl of the solution 24 h after the fourth moult

As previously described[1] injection of 20 μg of LSD per animal results in about 80% of continuous development and 20% diapause induction In the parental generation the LD_{10} was 35 μg per animal

Among non-diapausing animals, different crosses were performed (Table 1) The normal partners were taken from the normal stock defined earlier

The first striking observation was that all these F_1 larvae were highly resistant to LSD with a LD_{10} higher than 60 μg per animal Second, 100% of the diapausing progeny of cross B, whether or not injected with 20 μg of LSD, died either at the end of the chrysalid stage or as malformed imagos (Table 1, B_1 and B_2, respectively)

Among diapausing animals, new crosses were performed (Table 1) Normal animals were taken from the progeny of the normal stock defined earlier The sensitivity of the F_2 larvae to LSD in the three types of crosses seemed very high the LD_{10} fluctuated between 5 and 10 μg per animal

Different effects resulting from a treatment by LSD are apparent in the progeny of treated animals an altered response to a 9-h photophase and the sensitivity to toxic effects of the drug Two types of toxic effects have been detected One is a short term effect measured through LD_{10}, the second is a long term effect apparent at the imaginal moult in diapausing animals

The progeny of treated males and females (Table 1, B_1 and B_2) die at the imaginal moult although the egg laying ability of parents and hatching percentage, which were measured during all these experiments were found to be normal

The early toxic effect of LSD follows a curious pattern with a LD_{10} of 35 μg for the parents, of more than 60 μg for the F_1 and 5–10 μg for the F_2 and the F_3

The high resistance to LSD of the second generation may be explained by a detoxification mechanism, consequence of the parental treatment, enhanced by a further LSD treatment which could explain the slight improvement of viability in column B_2 Analysis of the effect of LSD on diapause induction must take the existence of such a resistance mechanism in account

Table 1 Effect of LSD on diapause induction by a 9-h photophase

Parents		Treated		Not treated			Control (Ringer)
Total number		378		312			59
% diapausing		18		78			86
F_1	A treated ♂ × normal ♀		B treated ♂ × treated ♀		C normal ♂ × treated ♀		Control
	A_1	A_2	B_1	B_2	C_1	C_2	
Total number	77	111*	60	101*	91	128*	312
% diapausing	99	100*	25	97*	79	48*	78
F_2		A_1 ♂ × normal ♀			C_1 ♀ × normal ♂		C_2 ♀ × normal ♂
Total number		107			133		110
% diapausing		45			57		65

* When some animals were treated again, their total number and percentage of diapause are given (F_1 column 2)
Amount of LSD injected per animal was 20 µg The control value in F_1 is taken from the parents

The untreated progeny of treated males and females (B_1) show 25% of diapause induction only This 'parental effect' is only observed if both parents were treated and completely disappears after a 20 μg LSD treatment (B_2) Such an induced resistance to LSD is asymmetric If only one of the parents is treated and if this parent is the male, then treatment of the progeny has no effect (A_1/A_2) If this parent is the female, then a slight shift toward photophase resistance after LSD treatment can be observed (C_1/C_2)

We must then conclude that the resistance mechanism observed in the F_1 is very efficient when transmitted by males (A_1) and less efficient when transmitted by females (C_1) The LSD triggered enhancement of the resistance to the drug seems to be transmitted only by the males (A_2 and B_2) and is only apparent when a parental effect is present (B_1/B_2)

This parental effect can be observed in the F_2 generation even if only the grandfather or the grandmother was treated with LSD (results are statistically different from the normal 80% of diapause induction with less than 1% chance of error) We are apparently able to observe this transmission because the resistance mechanism, which in the F_1 masked the parental effect, is absent in the F_2

The most probable target for LSD is the genetic material present in the germinal cells of the parental generation Successive crosses with normal animals lead to a dilution of treated genetic material and a progressive shift toward a normal situation

Damage to chromosome structure after LSD treatment has been described in different animals such as mice, humans and amphibians[2-6]. Unfortunately such damage is difficult to detect in Lepidoptera.

If what we have found in an insect is also true in mammals then we should observe a high resistance to LSD in the progeny of treated parents and physiological effects detectable even several generations after treatment could be predicted as these are common features of the different crosses we have performed. This could be confirmed in mice.

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Regions of the freezing curve causing changes in structure and viability of ram sperm

WHEN ram spermatozoa are chilled and frozen, their acrosomes are damaged even in the presence of protective substances in the diluent. It has been suggested[1] that these structural changes contribute to the reduced fertility of frozen ram semen. Although the motility and structure of spermatozoa are affected to different extents by freezing it is not known if these changes occur simultaneously or are caused at different stages of the freezing process.

The present work was designed to identify the critical regions of the freezing curve by assessing the motility of and acrosomal damage to spermatozoa after cooling to various temperatures below zero. The experimental design was a 4^2 factorial array of temperature to which the sample was cooled (−5° C, −15° C, −45° C and −196° C) and level of egg yolk in the diluent (0%, 0.15%, 0.75% and 3.75% v/v). Samples of ram semen were diluted in a buffered glucose solution similar to that described previously[1] except that the glucose content was increased from 185 mM to 247 mM. The semen was diluted at 30° C and then cooled at a constant rate of 0.21° C min^{-1} to 5° C. An equal volume of diluent containing glycerol was added at 5° C and the diluted semen was held at this temperature, for not less than 5 h. The final dilution rate was 20-fold. Semen was frozen in plastic straws (0.25 ml, Cassou, l'Aigle) at a constant rate of 12° C fall min^{-1} to −55° C in a controlled freezing device. For lower temperatures the straws were transferred at −55° C to liquid nitrogen (−196° C). The order of the freezing treatments in successive replications of the experiment was determined according to a Latin square design. By this means, the main effect of freezing treatments was separated from the possible influence of differences in the equilibration time at 5° C.

The samples were thawed 10 min after reaching the final temperature. All samples were examined under the microscope within 5 min of thawing, and motility was estimated on a subjective scale of 0–4 (ref. 2) and the percentage of motile spermatozoa was estimated to the nearest 10%. Smears of frozen–thawed semen were fixed, stained and scored as described by Watson and Martin[1], who scored acrosomal damage on a 0–3 scale (0 for an undamaged acrosome and 3 indicating loss of the acrosome).

The results and statistical analyses are shown in Table 1. A significant decline in the motility and percentage of motile cells after cooling and warming was seen in those samples cooled to −15° C or below, compared with those cooled only to −5° C ($P < 0.001$). Crystallisation of the external medium occurred at −12° C. Samples cooled to −5° C were therefore supercooled but not frozen; all other samples were frozen. This probably accounts for the reduction in survival of frozen samples since no difference in survival rate was detected between samples cooled to −15° C, −45° C and −196° C. Spermatozoa frozen to −15° C or below showed a greater mean acrosomal deterioration than those supercooled to −5° C ($P < 0.01$), but freezing to −45° C or −196° C was less detrimental than freezing to −15° C ($P < 0.001$). The greatest changes in the acrosomes were seen in those spermatozoa frozen to −15° C. Damage to acrosomes did not result from crystallisation of the suspending medium, however, since the degree of damage was similar in the samples cooled to −5° C and −196° C. Dehydration of the cells with consequent salt concentration, and intracellular ice formation and recrystallisation could all be factors contributing to the damage since these events could occur during the 10 min pause before rewarming. They are less likely to occur at lower temperatures because of the lower energy state of the sample.

Table 1 Mean survival and acrosome scores for ram spermatozoa cooled to different temperatures in diluents containing various levels of egg yolk

	Treatment	Motility (Scale, 0–4)	% Motile	Acrosome score (Scale, 0–3)
A	Temperature to which cooled (° C)			
	1 −5	2.44	45.94	1.11
	2 −15	1.86	30.94	1.51
	3 −45	1.63	27.34	1.23
	4 −196	1.80	30.47	1.12
B	Egg yolk level (%)			
	0	1.42	22.19	1.32
	0.15	1.92	32.50	1.30
	0.75	2.28	43.44	1.22
	3.75	2.09	36.56	1.13

Summary of analyses of variance

	Source of variation	Degrees of freedom	Variance ratios Motility	 % Motile*	 Acrosome score
A	Cooling treatments				
	1 against 2, 3 and 4	1	37.93†	58.04†	8.75‡
	2 against 3 and 4	1	1.62	1.20	29.13†
	3 against 4	1	1.62	2.20	2.63
B	Egg yolk				
	Egg yolk against none	1	37.93†	54.77†	3.00
	Levels linear	1	1.62	2.37	5.50§
	quadratic	1	5.52§	15.86†	<0.01
	Equilibration	3	1.03	0.36	2.13
C	Ejaculates¶	7	4.14†	10.43†	3.13‡
	Pooled first-order interactions of treatments	51	0.49	0.80	1.19
Residual (error) variance		60‖	*0.29*	*43.27*	*0.08*

* Data transformed to angles for analysis.
† $P < 0.001$; ‡ $P < 0.01$; § $P < 0.05$.
¶ 2 ejaculates were used within each replication row of the Latin square.
‖ 63-3 (for equilibration effect) degrees of freedom for 'error'.

The presence of egg yolk in the diluent improved the survival of spermatozoa after cooling and warming ($P < 0.001$). Survival with 0.75% egg yolk was significantly greater than with either the higher or the lower level of egg yolk ($P < 0.05$—motility score; $P < 0.001$—percent motile score). Acrosomal damage was decreased by increasing levels of egg yolk ($P < 0.05$). More egg yolk was thus required to protect the acrosomes, than to preserve motility. It may be that the stresses induced by chilling and freezing in the large surface area of the membranes surrounding the head of the spermatozoa are greater than those in the region of the midpiece. If a membrane-coating hypothesis is advanced for the protective action of egg yolk, the more labile membrane structures of the acrosome may require a greater concentration of egg yolk in order to stabilise them than do those involved with the movement of the spermatozoon. A similar pattern of response to the egg yolk levels was shown by the samples cooled to −5° C, to that seen in those cooled below the freezing point, since the 'cooling temperature × egg yolk level' interaction was non-significant for all scores. This supports the view that egg yolk plays a protective role during and after freezing similar to that before freezing.

Bank and Mazur[3] discussed the fact that there is no simple correlation between the survival and the degree and type of structural alteration of cells during freezing. For ram spermatozoa under the conditions in this experiment, motility was mainly affected by the phase change occurring near to −12° C, but the critical region, in which structural changes occurred, extended from this temperature to about −45 °C. It may well be that the freezing curve which allows maximum survival with minimum structural alteration has different cooling rates for different regions of the curve.

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Proteoid roots are microbially induced

ALTHOUGH some workers[1] have examined environmental factors affecting the formation of dense clusters of rootlets ('proteoid roots') in many taxa of the Proteaceae, the causal agents have not been elucidated. The basic phenomenon is an intense, localised production of short lateral roots occurring particularly in nutritionally poor conditions. Purnell[2] and Lamont[3] were unable to detect bacteria, actinomycetes or fungi in newly formed proteoid roots although microorganisms were sometimes found in older roots. Here we demonstrate that proteoid roots are caused by non-infecting rhizosphere microorganisms.

Nine hundred gram lots of two soils poor in nutrients (a podzolised soil, Mount Burr sand, South Australia[4] with 3 p.p.m. sodium bicarbonate extractable P; and a lateritic podzolic soil from Dwellingup, Western Australia[5] with less than 1 p.p.m. extractable P) were wetted to 70% field capacity with distilled water and placed in lidded polythene pots. Watering tubes of polythene tubing (1 cm diameter) with the lower end buried 2 cm into the soil were sealed into the lid, and the open end was covered with a metal cap. The assemblies were sterilised by γ irradiation (5 Mrad, Minster Carpets, Dandenong, Victoria). Seeds of *Banksia grandis* Willd. were surface sterilised and germinated on a nutrient agar[6]. Two sterile seedlings were planted in each pot by inserting the radicles through slits cut aseptically in the lid of the pot, and then sealing with petroleum jelly. Microbial inoculum was prepared by shaking 10 g of fresh proteoid roots (with attached rhizosphere soil) of *B. grandis* from a nutrient-poor soil in 200 ml sterile distilled water and 1 ml of this suspension was introduced through the watering tube to each 'inoculated' pot and 1 ml of sterile distilled water to uninoculated controls. Watering throughout the study was with sterile distilled water introduced by the capped water tubes. The plants were randomly distributed in a controlled environment growth cabinet and harvested after 4 months' growth at 20° C, with a 12 h day with light from Osram mercury vapour lamps (MBFR/u, 400 W, General Electric Company, London), providing 20,400 lumen m^{-2}.

In Mount Burr sand, none of the plants in the 12 sterile control pots produced proteoid roots while the plants in all 12 of the inoculated pots did; proteoid roots were particularly marked around the point of inoculation. In the lateritic podzolic soil none of the plants in the nine replicate control pots which remained sterile formed proteoid roots while all plants in the 12 inoculated pots did. The plants in the three microbially contaminated pots produced proteoid roots. None of the proteoid roots sectioned from inoculated pots was infected and we conclude that growth of microorganisms in the rhizosphere triggered formation of proteoid roots by the plant. Some microorganisms are known to produce growth substances affecting root morphology after infection by them[7,8] but this seems to be the first report of a major change in root morphology by non-infecting rhizosphere microorganisms.

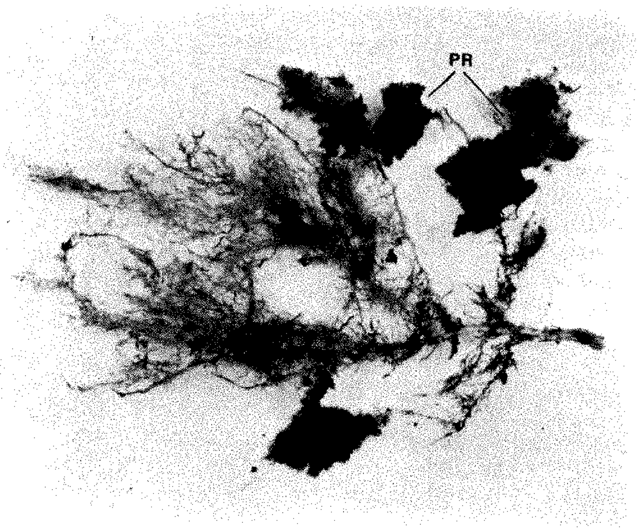

Fig. 1 Clusters of proteoid roots (PR) *Banksia grandis* (× 0.355).

Direct microscopic observation of proteoid roots from inoculated pots following staining[9] showed the rhizosphere to be dominated by bacteria. Counts of total bacteria, total aerobic viable bacteria, pseudomonads[10], Gram-negative bacteria[11], and *Bacillus spp.* (after heating dilutions for 10 min at 80° C) showed the rhizoplane numbers and frequency of these groups to be similar per mg of root with proteoid roots and corresponding non-proteoid roots on the same plant. Thus, the above counts did not give any suggestion of the identity of the causal organisms. Pure culture studies are proceeding. The production of proteoid roots in three pots with contamination infers that either a very common airborne organism or a wide range of organisms could induce proteoid root formation. If specific organisms are involved, however, it is not impossible that a few of them survived the γ-irradiation treatment.

Dry weight of shoots of plants with proteoid root and without proteoid roots respectively were 1.22 g and 0.67 g in Mount Burr sand (difference significant at $P<0.01$) and 1.40 g and 0.79 g in the lateritic podzol (difference significant at $P<0.01$). In each soil the weights of the roots of plants with proteoid roots and plants without proteoid roots were almost identical. Proteoid roots accounted for approximately half the root weight in Mount Burr sand and approximately 40% of the root weight in the lateritic podzolic soil. Jeffrey[12] demonstrated the importance of dense surface mats of proteoid roots trapping sudden phosphate accessions and also attributed an increased uptake of phosphate from solution culture to their greater absorbing surface. Figure 1 indicates the intense exploration of soil afforded by proteoid roots, and this would be expected to enhance uptake of poorly mobile ions already in the soil[13] as well as trapping added nutrients. Baylis[14] and Bowen[15] have commented on the importance of mycorrhizal associations in compensating low root density in uptake of poorly mobile ions from soil.

Uptake of ^{32}P from 5×10^{-6} M KH_2PO_4 in 5×10^{-4} M $CaSO_4$ over 15 min at 20° C showed uptake of phosphate by proteoid roots to be 1.82 ± 0.18 (s.e.) $\times 10^{-13}$ mol cm^{-2} s^{-1} compared with $0.48 \pm 0.07\times10^{-13}$ mol cm^{-2} s^{-1} for similar young unsuberised non-proteoid roots (differences significant at $P<0.01$). Assuming the microbial component of uptake to be similar (as indicated by the similar microbial counts and composition above) the enhanced phosphate absorbtion by proteoid roots is due not only to their greater surface area but also to increased absorbing ability. Stimulation of phosphate uptake and translocation by noninfecting rhizosphere microorganisms has been suggested for other plant groups[16,17].

We see the results as being significant in demonstrating yet another system which plants have evolved for enhancing their nutrition in low nutrient soils, not by themselves but in association with soil microorganisms. Other, better known, examples are legume and nonlegume nitrogen fixation[18] and various types of mycorrhizas[19] increasing ion uptake. Lamont[1] demonstrated experimentally that low or unbalanced N and P status of the growth substrate enhances proteoid root formation in nonsterile conditions. Our demonstration that rhizosphere microorganisms induce proteoid root formation suggests a need to examine the extent to which observed differences between soils in proteoid root formation are due to soil microbiological differences rather than soil chemical and physical differences.

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Oxygen microenvironment and respiratory oscillations in cultured mammalian cells

OXYGEN transport in cultures of attached mammalian cells depends on the kinetics of gas diffusion through the overlaying medium. Working with liver parenchymal cells, Stevens[1] calculated that the oxygen requirements and energy demands of a monolayer culture could not be met by atmospheric pO_2 if the fluid overlay was deeper than 0.34 mm. McLimans[2] theorised that under the standard medium overlay of 1.5–5.0 mm, oxygen depletion by a respiring monolayer of liver parenchymal cells would exhaust the atmospheric oxygen supply at the plane of the attached cell sheet within 35 min. His model suggests that adaptive growth of cultured cells occurs only when respiration does not exceed the delivery of oxygen to the monolayer.

To test these theories and the validity of arguments linking oxygen availability with respiration, energy metabolism and the regulation of cell growth, we constructed microoxygen cathodes[3,4] and investigated microenvironmental pO_2 in the medium overlaying attached cultures of WRL-10A cells. This subline of the L-cell mouse fibroblast, isolated in our laboratory[5], was used because we already had information about changes in medium pO_2 and respiratory rates during regulated states of growth and differentiation of these cells in suspension cultures[6,7]. Cells were grown attached to optically flat 50 mm Petri dishes with 6 ml of Eagle's minimum essential medium (MEM) supplemented with 20% horse serum, antibiotics and glutamine. Dishes were placed on the stage of an inverted microscope inside an environmentally controlled chamber, and maintained at 35° C in a humidified atmosphere of 5% CO_2 and air. A Pt/Ir oxygen microcathode with a tip 1–3 μm in diameter and a corresponding resolution 6–20 μm in diameter, was secured in a micromanipulator clamp, and positioned in the centre of the objective field so that descent of the cathode could be observed. An Ag/AgCl reference anode placed in a saline well adjacent to the dish was joined to the medium by an agar bridge. Starting at the fluid–air interface, the cathode was

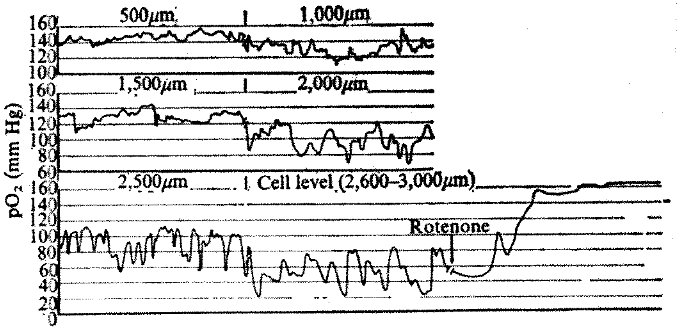

Fig. 1 Oscillations of microenvironmental pO_2 in the medium overlaying attached cultures of 10^7 cells per 50 mm Petri plate were measured amperometrically with calibrated Pt/Ir microoxygen cathodes polarised between 0.6 and 0.8 V. The oscillations were traced on a strip chart recorder connected to an electrometer which was attached in series to a cathode and a Ag/AgCl reference anode. The current output of these sensitive cathodes ranged between 0.5×10^{-11} and 0.5×10^{-12} A per mm Hg. Distances measured in micra represent depths from the surface of the medium towards the attached cell sheet. Oscillations at each depth are presented in comparable 30-min segments.

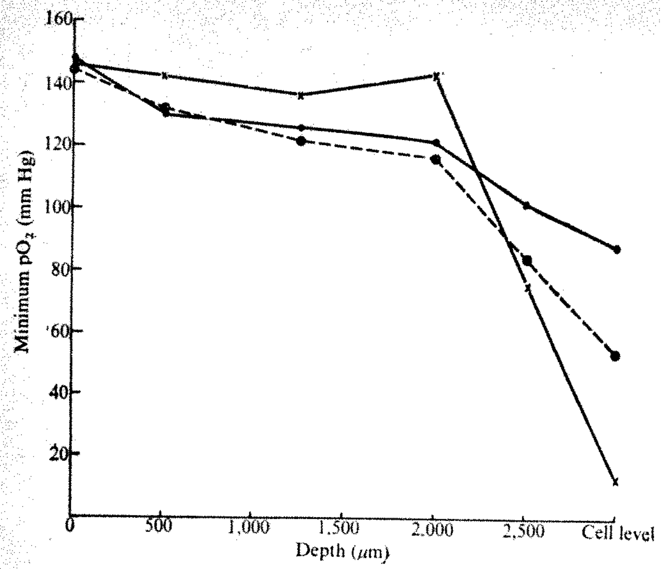

Fig. 2 Density-related oxygen gradients in the medium overlaying populations of attached WRL-10A cells. Experimental cultures contained 5×10^6 (●——●), 10×10^6 (○ - - - - ○), and 16×10^6 (×——×) cells per plate. Minimum pO_2 values represent means of the lowest pO_2 readings obtained from all oscillations recorded at each indicated depth.

lowered through the medium towards the attached cell layer and pO_2 changes were measured polarographically at selected depths. Pilot experiments detected the existence of oscillating concentration gradients of oxygen above the attached cell sheet. Because this oscillatory phenomenon had neither been predicted nor described in any literature on mammalian cell cultures we have tried to describe these dynamic oxygen fluctuations in detail, to determine their origins, to establish their relationship to cell density and to derive from them clues about oxygen availability as it relates to mechanisms controlling respiratory activity in attached populations of exponentially proliferating cells.

Using populations of 10^7 cells per plate, continuous recordings were made of the pO_2 oscillations at specific depths in the overlaying medium. A composite of the collected data (Fig. 1) shows that oscillation amplitude increased with depth. At 500 μm the oscillations were very shallow, at 1,000 μm and 1,500 μm they were slightly larger, but still relatively small while at 2,000 μm and 2,500 μm oscillations were mixed large and small. Continuous undampened oscillations with major and minor peaks occurred at cell level and their mean peak frequency ranged from 0.34 – 0.56 per min in different experiments. At each increasing depth, oscillations occurred within a characteristic range of progressively decreasing minimum and maximum pO_2. For example, at 500 μm they occurred between 140 and 160 mm Hg, while at the cell level they occurred between 20 and 80 mm Hg. Correspondingly, conditions of minimum O_2 availability at the various depths are described by a mean minimum pO_2 gradient, that is an oxygen gradient based on the average of the lowest pO_2 readings obtained from all the oscillations recorded at each particular depth. After recording the pO_2 oscillations at the cell level for 30 min (Fig. 1), 50 μl of a rotenone solution, 10^{-8} M in ethyl alcohol, were added to determine whether the oscillations were related to cellular respiratory activity. The effect of this respiratory inhibitor[8] was an immediate cessation of oscillations, followed by 9 min of maintained low pO_2, a last oscillatory rise and fall of local oxygen tension and a subsequent steady increase in pO_2. This brought the microenvironmental oxygen tension back into sustained equilibrium with atmospheric oxygen. Having thus demonstrated that the oscillations are an expression of cellular respiratory activity with oxygen depletion due to cell respiration and oxygen replacement due to diffusion, we investigated the effect of cell density on mean minimum pO_2 gradients.

Replicate cultures seeded with 7.0×10^5 cells per Petri plate were maintained by daily medium renewal and analysed polarographically at cell densities of approximately 5, 10 and 16×10^6 cells per plate. Comparisons of pO_2 gradients (Fig. 2) showed that from the air–fluid interface down to 2,000 μm, all three cell densities had media oxygen tensions that were relatively high and nearly in equilibrium with atmospheric pO_2. From 2,000 μm down towards the cell level, however, the oxygen tension decreased sharply in a directly inverse relationship to increasing density. Thus, the negative slope of the 5×10^6 cell population is the least steep and the slope of the 16×10^6 cell population is steepest. Likewise the mean minimum pO_2 at the cell level also decreased in direct proportion to increasing cell density, with the value of the 5×10^6 cell population reaching approximately 100 mm Hg, the 10×10^6 cell population reaching about 60 mm Hg, and the 16×10^6 cell population reaching about 19 mm Hg. Thus in attached cultures oxygen availability and microenvironmental O_2 gradients are directly related to the density and combined respiratory activity of the entire cell population.

When the amplitudes of the pO_2 oscillations were measured and their mean values at increasing depths compared (Fig. 3), the following correlations appeared. In cultures of 5 and 10 million cells the mean oscillation amplitude increased with depth down to 2,500 μm. At each depth the mean oscillation amplitude for the 10^7 cell population was significantly greater than the corresponding amplitude of the 5×10^6 cell population. Amplitude therefore increased as a function of both depth and increasing density. At densities of 16×10^6 cells and greater, the combined respiratory activity of the larger population intermittently reduced the microenvironmental oxygen supply at the cell level to extremely low levels, at which the respiratory activity seemed to be affected by oxygen limitation. Oscillatory behaviour in these high density populations is under investigation.

Our results indicate that the respiratory activity of WRL-10A cells under the condition of these experiments is periodic rather than constant. Furthermore, the density-dependent nature of

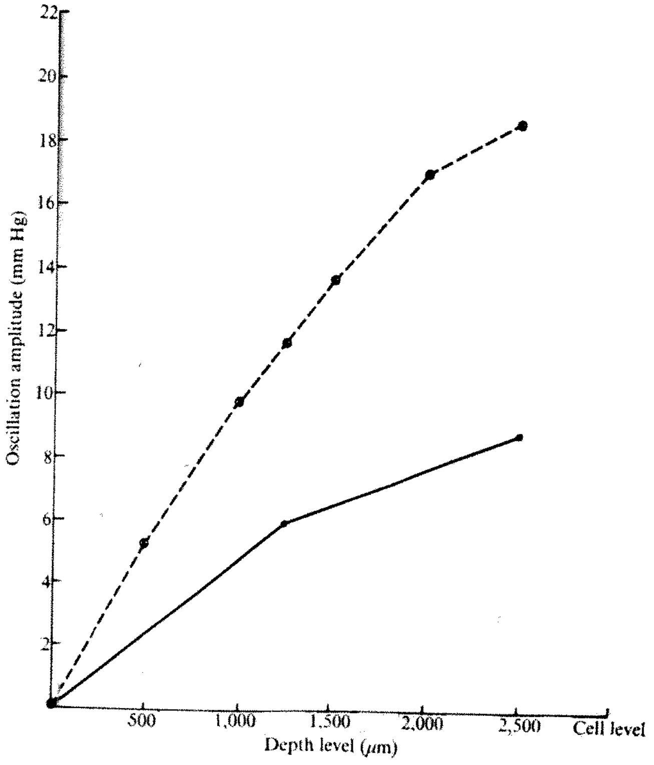

Fig. 3 Amplitudes of oscillations in exponentially growing cultures with 5×10^6 (●——●), and 10^7 (○ - - - ○) cells per plate. The values shown represent the means of all oscillations, large and small, recorded at each indicated depth.

the oscillations and of the media pO_2 gradients suggests that the entire cell population, or at least very large portions of it, respires in synchrony. This is evident because individual cells respiring periodically but out of phase, would tend to dampen or cancel out the oscillations. In the cellular microenvironment, the succession of predominantly undamped oscillations with minimum pO_2 values above 15 mm Hg reflects both the continuous cycles of partial depletion and renewal of microenvironmental oxygen and a control of respiratory activity that cannot be ascribed to mere periodic fluctuations of oxygen availability. We suggest therefore that cellular respiration in these cultures responds to environmental feedback[9] that is subject to modification by local exhaustion and diffusion of essential nutrients and metabolites and can alternately and synchronously turn the respiratory system on and off in a large number of cells. Such an oscillating system might consist of an energy balance between AMP, ADP and ATP or a fluctuation in some oxidisable substrate pool such as NADH, or a matrix of these and other interdependent variables such as pO_2, *p*H, pCO_2, glucose, Na^+ and K^+ all working in concert to control respiration and growth metabolism[10,11].

Stoker[12] demonstrated that populations of attached 3T3 cells, having reached confluency and stopped growing, can be induced into highly localised growth by gentle local agitation of the supporting medium. His inference is that the microenvironmental diffusion barrier proposed by Rubin and Rein[13] was reduced by the local disturbance thus changing the concentrations of essential growth factors or inhibitors and stimulating localised mitotic activity. Our data have provided direct demonstration of localised dynamic changes in the constitution of the growth media of attached cultures. The proposition that such changes may play an important role in growth regulation will be tested by extending these investigations to 'contact inhibited' cells.

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Developmentally regulated cell surface alterations in *Dictyostelium discoideum*

THE life cycle of the cellular slime mould, *Dictyostelium discoideum*, can be divided into two mutually exclusive phases[1]. The vegetative state, in which the cells divide by fission every few hours, is characterised by the absence of intracellular communication; whereas the developmental social state, which is initiated by the exhaustion of nutrients, exhibits many forms of such interactions. These include the ability to respond to and relay chemotactic signals[2], and to form stable species-specific cell to cell contacts[3-5]. The ability to form stable cell contacts can develop in the absence of a chemotactic gradient, as demonstrated by the immediate formation of normal aggregates upon plating of cells which have been maintained in suspension in a nutrient-free salt solution for 8–9 h (ref. 1). New antigens appear and function on the cell surface during development[3,4]. Gerisch and his coworkers have shown that univalent antibodies directed against the surface of 12 h cell aggregates interfere specifically with the formation of the stable cell to cell contacts[4-6]. Furthermore, it has been demonstrated that a developmentally controlled, heat-stable component, which is present in membrane preparations from 12 h aggregates, interferes specifically with the morphological and biochemical development of the organism (J.E.S., and J. Tuchman, unpublished, and J. Tuchman *et al.*, unpublished).

Most studies on cellular components which reside on the outer cell surface of the plasma membrane are complicated by the fact that the cell surface membrane must be disrupted in order to be purified and characterised. For some molecules on the outer cell surface this complication can be avoided by selectively labelling them before disruption of the cell[7,8].

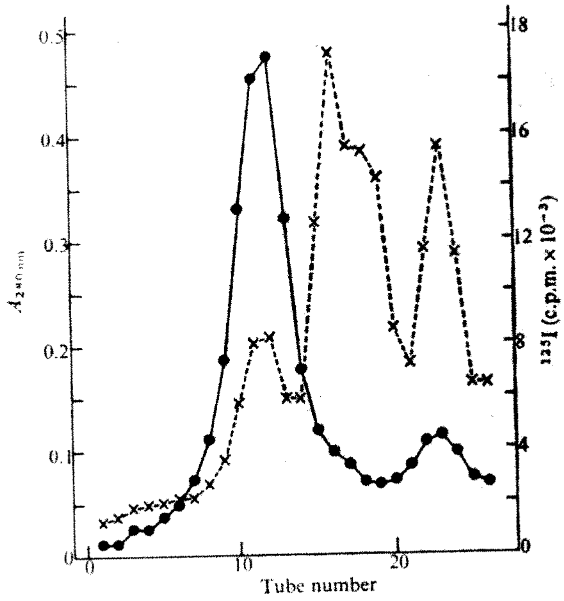

Fig. 1 Sucrose gradient profile of subcellular fractions of ^{125}I-labelled *D. discoideum* cells have formed firm aggregates 12 h after nutrient deprivation and plating. Cells were suspended at 10^8 cells ml^{-1} and carrier-free $Na^{125}I$ usually to a concentration of 400 μCi ml^{-1}. The reaction was initiated by the addition of lactoperoxidase (E.C. 1. 11. 1. 7) (Calbiochem) and glucose oxidase (E.C. 1.1.3.4) (Worthington Biochemicals) to final concentrations of 20 μg ml^{-1} and 0.1 units ml^{-1}, respectively. The reaction was allowed to continue for 10 min at room temperature with occasional swirling. The reaction was stopped by addition of phosphate buffered iodide, PBI (phosphate buffer saline with the NaCl replaced by NaI), and the cells were pelleted through a cushion of PBI + 10% glycerol. The resulting pellet was resuspended in 1 ml of PBS and frozen at 70° C. After thawing of the cells (which resulted in cell lysis), membranes were prepared by layering the suspension over a linear gradient of 0.5–2.0 M sucrose, 0.02 M Tris, *p*H 8.0, 0.005 M $MgCl_2$. The gradients were centrifuged at 23,000 r.p.m. for 18 h at 4° C in a MSE SW 6 × 14 rotor. ●, ^{125}I (c.p.m.); ×, A_{280nm}. The membrane containing turbid band was diluted with at least five volumes of 0.02 M Tris, *p*H 8.0, 0.005 M $MgCl_2$, and centrifuged at 30,000 r.p.m. for 30 min at 4° C in an MSE SW 6 × 14 rotor.

We used the Na^{125}I-lactoperoxidase–glucose oxidase coupled system[9] to monitor some of the developmentally controlled changes on the cell surface of *D. discoideum*. This method selectively labels proteins which have a tyrosine exposed to the extracellular aqueous environment. We observed that the plasma membrane of *D. discoideum* in its vegetative growth phase contains two major proteins (molecular weights about 135,000 and 55,000) which are on the external surface and accessible to labelling by the ^{125}I-lactoperoxidase system. After the cells have progressed through their developmental phase to the point of forming firm aggregates a new major protein (molecular weight about 130,000) becomes accessible to labelling. Other less prominent labelled proteins show quantitative changes in their availability to this reagent. *D. discoideum*, strain Ax-2, an axenic haploid strain[10], was grown to mid-log phase 3–5×10^6 cells ml^{-1}) in HL-5 medium[11]. The cells were collected and washed free of nutrients in cold pad diluting fluid (PDF)[12], and 5×10^7 cells were deposited on black Millipore filters (4.25 cm). After proceeding through their normal developmental phase for varying periods of time, the cells were harvested in cold PDF, washed once with phosphate buffered saline (PBS), and then with PBS+5 mM glucose. The cell surface was then labelled with ^{125}I (carrier-free Na^{125}I, Radiochemical Centre, Amersham, Bucks) using the Na^{125}I-lactoperoxidase–glucose oxidase coupled system as described in Fig. 1.

The labelled cells were lysed and fractionated on a linear sucrose gradient, as shown in Fig. 1. The lowest band in the gradient contains practically all of the TCA precipitable ^{125}I counts. This turbid band, when viewed under a phase contrast microscope, or by electron microscopy, is seen to consist of large membranous fragments, as well as smaller vesicular components[13]. Whereas longer centrifugation results in further sedimentation of the other bands, the position of the turbid ^{125}I-containing band is not affected, and it is, therefore, assumed to be at its isopynic density (about 1.16–1.17 g ml^{-1}) (J.E.S. and J. Tuchman, unpublished).

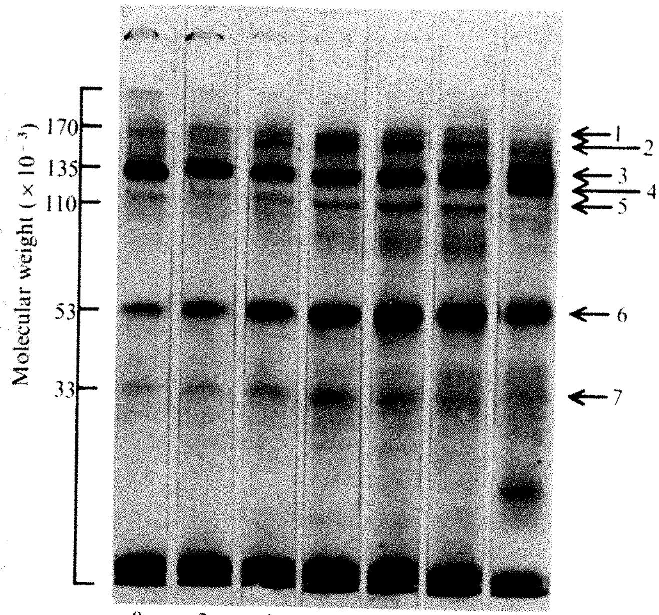

Fig. 2 Autoradiogram of 7.5% sodium dodecyl sulphate-polyacrylamide slab gel electrophoretogram of ^{125}I-labelled membranes of *D. discoideum* at various stages of normal differentiation. Membranes were dissolved in sample buffer containing 1% SDS 0.01 M dithiothreitol, 10% glycerol, and 0.05 M Tris, *p*H 6.8, by boiling for at least 2 min. Electrophoresis was done in slabs where 12 samples could be run in parallel on the same slab[16]. The buffer system used was that of Laemmli[17]. Electrophoresis was done at 50 V for about 1 h, until the bromophenol blue marker entered the separation gel, and then at 100 V (about 10 V cm^{-1}) until the marker reached the bottom. The slabs, after they were stained with Coomassie blue if required, were dried down on to paper, and autoradiographs made on Kodirex X-ray film.

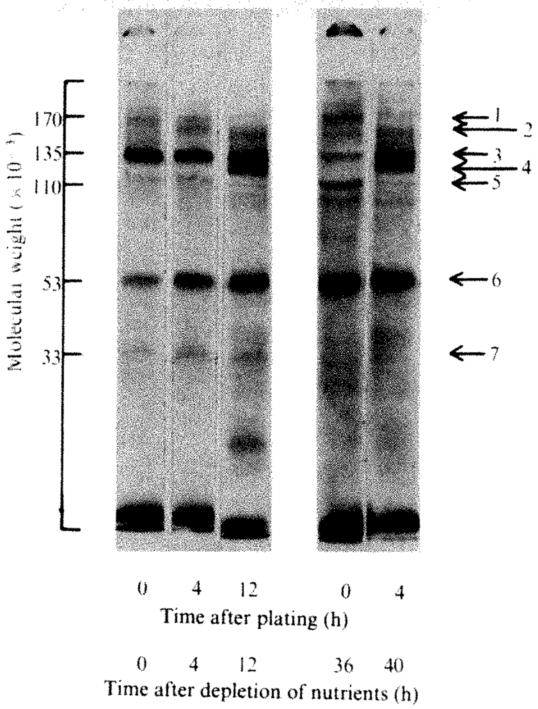

Fig. 3 Autoradiogram of 7.5% SDS-polyacrylamide slab gel electrophoretogram of ^{125}I-labelled membranes of *D. discoideum* at various stages of normal differentiation or at various stages of development of 'aggregation competence' in suspension culture. Samples for suspension culture were collected as described and resuspended at 10^7 cell ml^{-1} in PDF. The cells were maintained for various periods of time in shaking culture. In the above experiment the cells were maintained in liquid suspension culture for 36 h at which time one aliquot of 10^8 cells was labelled with ^{125}I. An identical aliquot was plated on black Millipore fitters and allowed to form firm aggregates (3–4 h after plating), before labelling.

In the absence of lactoperoxidase and glucose oxidase, the labelling is only 0.6% of the control level, suggesting that the reaction is totally dependent on the exogeneously added enzymes, which presumably do not enter the cell. Removal of 94% of the TCA precipitable counts by mild trypsinisation of whole cells (leaving the cells intact) also supports the conclusion that the label is only attached to proteins on the cell surface. If cells which have been mildly trypsinised after labelling are fractionated by sucrose gradient centrifugations essentially all of the remaining TCA precipitable counts are found with the soluble proteins. Furthermore, if the cells are lysed (freeze–thaw) before the labelling procedure, the ^{125}I counts are found to parallel closely the $A_{280\,nm}$ profile in the sucrose gradient, and SDS polyacrylamide slab gel electrophoretic analysis of such preparations shows that the protein banding patterns obtained by staining with Coomassie blue or by ^{125}I autoradiography are essentially identical. These observations indicate that the reaction labels only a discrete portion of the cellular proteins of intact cells, that this selectivity is dependent on the integrity of the cells and that all of the labelled proteins reside on the outer surface of the plasma membrane where they are accessible to trypsin.

During the first 12–14 h of normal differentiation (after depletion of nutrients) *D. discoideum* proceeds from unicellular amoebae to a multicellular organism composed of approximately 5×10^5 cells (ref. 14). At any point during these early stages of development the organism can be readily collected and mildly disrupted into a single cell suspension[15]. If intact cells of such samples are labelled with ^{125}I and their isolated membranes analysed by SDS-polyacrylamide gel electrophoresis, the results shown in Fig. 2 are obtained. For convenience, seven of the more noticeable ^{125}I-containing bands are numbered. It is interesting that none of these seven labelled bands (with the possible exception of band No. 6) comigrates with any of the major Coomassie blue staining bands of the same preparations. Although quantitative changes can be seen in several of the labelled proteins, the most noticeable change is the appearance of band No. 4 between 10 and 12 h after plating. At this point in the morphogenetic pathway, *D. discoideum* has just formed firm pointing aggregates. Up to 10 h after plating there is an apparent increase in the labelling of bands Nos 2, 5, and 6; whereas, the labelling of band No. 3 seems to decrease over this period.

The initial triggering event for the shift from the unicellular state to the multicellular state is the exhaustion of nutrient supply[14]. Thus, the zero point for the timing of developmental steps in *D. discoideum* is quite easily established experimentally. Gerisch has shown that the functional state of cells maintained in suspension in a nutrient free medium changes; after 8–9 h they become capable of forming aggregates immediately after plating[1]. These aggregation competent cells, like normal aggregating cells, show an elongated morphology and a preference for end-to-end contacts. Furthermore, they express an antigen(s) which is present on the surface of aggregating cells, but not on growth phase, single cells[4]. As shown in Fig. 3, *D. discoideum* can be maintained in suspension culture for at least 36 h without the appearance of the ^{125}I-labelled band No. 4. The same result is obtained for suspension cultures maintained for shorter periods of time, for example 16 h. It is interesting that in these conditions, as in normal development, the amount of labelling of bands Nos 2, 5 and 6, seems to increase while that of band No. 3 decreases considerably. Moreover, if these same cells are plated to allow them to form normal aggregates, the labelling of band No. 4 appears very rapidly and coincident with the morphological development of firm pointing aggregates.

Thus, in our conditions the acquisition of 'aggregation competence' in suspension cultures is not accompanied by any major qualitative change in the iodinatibility of cell surface proteins, although interesting quantitative changes do occur. Band No. 4 (which appears coincident with the formation of firm aggregates after about 12 h of normal development) is iodinatible only after the suspension culture cells are plated and allowed to form firm aggregates. Consequently, the iodinatibility of band No. 4 does not appear to be associated with the changes in cell surface molecules which are necessary for chemotaxis or aggregation competence in *D. discoideum*. These experiments show clearly that the availability of band No. 4 to this reagent is not due solely to the timing of independent metabolic events initiated by nutrient deprivation. Rather its iodinatibility seems to require participation of the cells in certain intercellular and morphogenetic interaction.

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Meiosis in *Dictyostelium mucoroides*

THE cellular slime moulds are favourable organisms for the study of morphogenetic processes. But so far, genetic analysis of their development has been hampered by the apparent absence of a true sexual cycle, although progress has been made in parasexual genetic analysis[1–3]. Recent ultrastructural observations of the macrocysts of two species have suggested, but not proved, that karyogamy and meiosis are an integral part of dormancy and germination of this developmental stage[4–6]. We present here genetic confirmation that in *D. mucoroides* (strain DM-7) the assortment of mutant traits among progeny of single macrocysts is consistent only with meiosis.

Macrocysts of DM-7 are postulated to contain at least one zygote. The fusion and karyogamy of two haploid cells of possible genotypes (*aB*) and (*Ab*) gives rise to a dihybrid of genotype (*AaBb*). Since DM-7 is homothallic, either parental genotype may fuse with itself as well, giving roughly equal numbers of uniparental and biparental macrocysts, on the assumption that there is one zygote per cyst[7]. In biparental cysts, the assortment of genetic factors among the four primary daughter nuclei of a meiosis results in a particular type of tetrad. Only three kinds of tetrad are possible from a single meiosis, regardless of the linkage relationships of the markers in the dihybrid nucleus[8]. They are the parental ditype (*aB*+*aB*+*Ab*+*Ab*), the non-parental ditype (*AB*+*AB*+*ab*+*ab*), or the tetratype class (*aB*+*Ab*+*AB*+*ab*). DM-7 is advantageous in that single macrocysts can be germinated wtih high efficiency and progeny samplings analysed for possible tetrad assortments..

Individual macrocysts possessing two or more zygotes could possibly arise through independent karyogamies, or by mitoses of a diploid zygote nucleus. These alternative mechanisms are distinguishable from the outcome of a single meiotic event (see below).

The progeny yield of DM-7 macrocysts often approaches 200 amoebae. Ultrastructural studies of germinating cysts have indicated that cytoplasmic cleavage is preceded by many nuclear divisions[6]. The timing of these apparent mitoses relative to a postulated meiotic event may influence the efficiency of this system for genetic analysis.

From these considerations and others, we predict that progeny of a single macrocyst will assort into one of only three tetrad classes, on the assumption that: (*a*) a single karyogamy followed by one (or more) meiosis generates all of the progeny; (*b*) there is no seriously detrimental mitotic or sampling selection against any single progeny

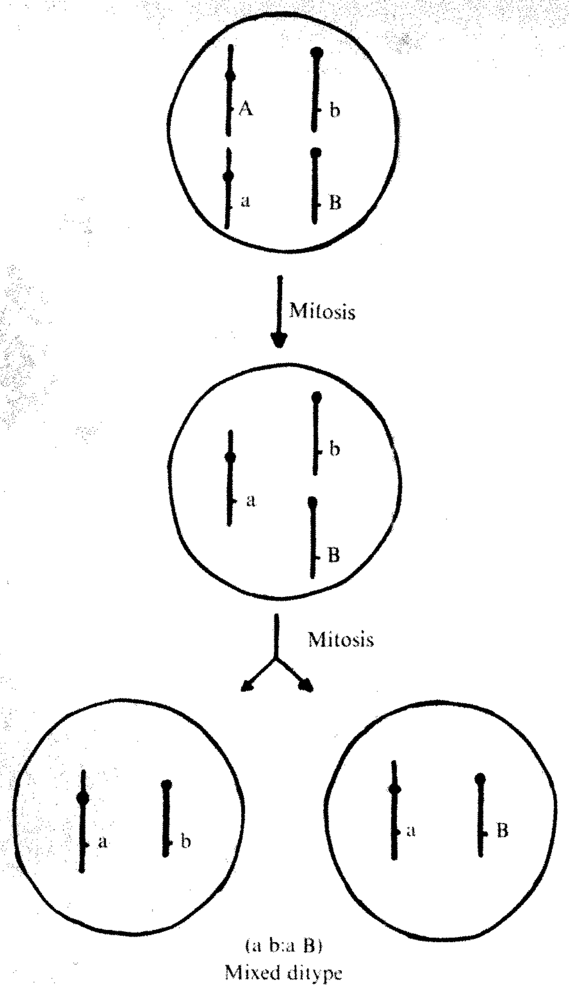

Fig. 1 Diagram of chromosomal losses associated with a hypothetical mitotic gene assortment mechanism in macrocysts. At the top is a dihybrid 'zygote' nucleus shown with only two chromosome pairs for clarity. An intermediate aneuploid stage during haploidisation, and two stable haploid nuclei are also represented. The mechanism of chromosomal loss is not depicted, whether by mitotic non-disjunction or by random cytoplasmic destruction. In the event that all daughter nuclei retain only two assortments of the markers of ditype will result. This outcome is most likely if one of the chromosomes bearing a mutant allele is lost at the first mitosis as indicated here. Otherwise, all copies of it must be lost from all daughter nuclei in successive mitoses. On a random basis six ditypes are equally probable, four mixed (for example, one shown above), one parental ditype and one non-parental ditype. The mixed ditype patterns of assortment do not occur in meiotic reduction division (see text).

genotype, and (*c*) there is no assortment through mitotic haploidisation.

Assumption (*c*) implies the possibility that within macrocysts some sort of accelerated parasexual mechanism could account for assortment patterns. Parasexual diploidy has been induced in *D. discoideum* under conditions similar to those used for macrocyst production^{3}. The dihybrid zygote could undergo haploidisation by chromosomal losses in successive mitoses^{9}. If such losses are random, single macrocysts would yield predominantly tetratype assortments but also several combinations inconsistent with meiosis (for example, Fig. 1). In the event that two genotypes arise from a single macrocyst, the parental and non-parental ditypes are just two of six equally probable ditype combinations (unlinked markers). So called 'tritypes' involving any three of the four segregant genotypes should also be recovered frequently. Thus recovery of mixed ditypes (Fig. 1) and tritypes together provides a simple test for the occurrence of mitotic haploidisation.

Table 1 Progeny samplings of single macrocysts for the cross *plq* × *cac*

Parental genotypes	Sample progeny clone phenotypes*				Macrocyst tetrad classification†	No. of macrocysts scored
(*plq*,+)×(+,*cac*)	(*plq*,+)	(+,*cac*)	(+,+)	(*plq*,*cac*)		
	145	0	0	0	PT (*plq*)	2
	0	398	0	0	PT (*cac*)	5
	34	37	0	0	PD	2
	0	0	16	19	NPD	1
	49	72	36	36	TT	6
Totals	228	507	52	55		16

Macrocyst production techniques were slightly modified from those worked out by Nickerson *et al.*^{10}. Cells of each genotype were collected and mixed before they became aggregation competent. Aliquots (0.25 ml) containing about 1.0×10^{7} cells of each genotype were deposited on 0.1% lactose, 0.1% peptone agar, the same medium as used for growth, but without additional bacteria. They were then placed in metal cans at 21° C to produce macrocysts. After storage for 22–35 d, the cross plates were removed from the container to initiate germination^{11}. Single macrocysts were picked and deposited in drops on to fresh, distilled water agar (2%). Drop transfers on the water agar effectively washed the macrocysts free of contaminating spores or amoebae. The germination plates were placed at 13°–15° C in a humid incubator, the interior illuminated by two 40 W incandescent tube bulbs. Significant germination began within 4 d and ultimately reached 60–80% of the transfers. Progeny of germinating macrocysts usually culminated in a small fruiting body on top of the cyst case. Blocks of agar with the cyst case, fruiting body and occasionally amoebal progeny were transferred to small aliquots of Bonner's salt solution^{13} containing suspended *E. coli*. The vortexed suspension was spread on to two PDB-5% agar plates per macrocyst and scored for clonal phenotypes after 3 d incubation at 28° C. Double mutant and wild type progeny clones were stable when sub-cloned on PDB-5% agar.

* Genotypes: (*plq*,+) = *plaque*, (+,*cac*) = *cactus*, (+,+) = wild type, (*plq*,*cac*) = the double mutant. The linkage relationships are undefined. The numbers are total progeny for the sum of the macrocysts scored.

† Tetrad classifications: PT, single parental type, PD, parental ditype; NPD, non-parental ditype; TT, tetratype class.

In this study, amoebae or spores were usually cloned on agar plates spread with *Escherichia coli* (B/r) for the screening of mutant strains and for analysis of the products of the genetic crosses. Clonal phenotypes of the three mutants selected for this study are shown in Fig. 2. The mutants *cactus*, *tag* and *plaque* each have an unusual morphological behaviour in one aspect of either clonal growth, aggregation streaming or fruiting body construction. In pairwise crosses each mutant phenotype is sufficiently distinctive to permit identification of all expected progeny phenotypes.

The crosses were initiated by mixing equal numbers of cells of each genotype under conditions leading primarily to macrocyst formation^{10}. Individual macrocysts were germinated according to the method of Nickerson *et al.*^{11} Contamination of the germinating macrocyst progeny by non-encysted parental amoebae or spores was eliminated by carefully controlling the conditions of macrocyst production, and after dormancy, by washing the cysts with drop transfers on the germination agar (H_2O). The lactose–peptone agar used for macrocyst production was dried overnight so that when aliquots of mixed cells (0.3 ml) were spread, a liquid film covering the aggregations persisted for only a few hours. Under prolonged wet conditions, the amoebae secrete large amounts of a slime material which traps cells or spores around the maturing macrocysts and prevents effective isolation and washing of the cysts. The complete set of progeny recoveries for the cross of *plaque* and *cactus* (*plq* × *cac*) is presented in Table 1. The numerical distribution of macrocysts classified as tetrads for all the crosses performed is given in Table 2.

The macrocyst progeny of *plq* × *cac* consistently fell into five classes (Table 1), three tetrad types and the two parental classes (selfed macrocysts). The reassortment of

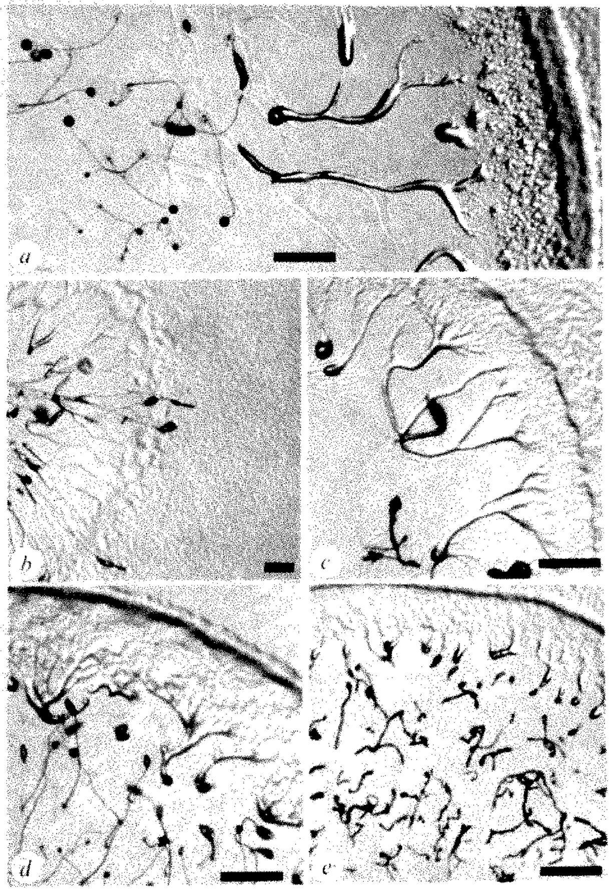

Fig. 2 Clonal morphology of DM-7 wild type and derivative strains (all bars represent 0.5 mm). The wild type stock and mutants were cultured with *E. coli* B/r on a buffered peptone–dextrose agar (designated PDB-2%)[8] at 21° C or 28° C. Mutagenesis procedures followed the method of Yanagisawa *et al.*[12], using nitrosoguanidine (Aldridge Chemical Co.). Surviving cells were initially regrown and screened on PDB with 5% replacing the 2% agar. The increased agar concentration restricts clonal spreading in the wild type (*A*) without affecting morphogenetic behaviour (screening efficiency 100+ clones per plate). *a*, DM-7 wild type. The clone boundary at right is the feeding front of amoebae on the bacterial lawn. Aggregation streams orient radially and are prominent, with complete fruiting bodies at the centre of the clone. *b*, The mutant clone *plaque* (*plq;* isolate 773–2). This mutant has a spreading feeding front on 5% PDB forming a 'halo' appearance around the clone, and prominent streaming, with complete though somewhat fragile fruiting bodies. *c*, Mutant *cactus* (*cac*; isolate 773–18). This mutant has a clone boundary and streaming like wild type, and complete fruiting bodies with excessively thickened stalks. *d*, Mutant *tiny aggregations* (*tag*; isolate 773–23). The clone boundary in this mutant is like wild type but aggregation size is much reduced in proportion to the clone size. Streams are radially polar and fruiting bodies are small, but complete. *e*, *cactus*, *tag*, a double mutant, which has reduced aggregation size characteristic of *tag* while stalk thickening indicates the cactus phenotype.

the two markers to produce wild type and double mutants in seven tetratype and non-parental ditype macrocysts conclusively establishes the existence of a dihybrid zygote stage (possible genotype: *plq*/+, *cac*/+). Second, significant numbers of progeny of each genotype were recovered from these macrocysts. Although the relative numbers are not always statistically equal, the suggestion is strong that there is no detrimental mitotic selection against any one of these genotypes during germination.

The mechanism of gene assortment, either meiotic or parasexual, is established from the data of Table 2. Of the 74 macrocysts germinated in the four crosses, 22 were classified as parental ditypes, non-parental ditypes or tetratypes. Each biparental macrocyst yielded a progeny genotype assortment in one of only the three normal kinds of tetrads, as expected for meiotic behaviour. Of particular interest are the ditypes; among 22 biparentals none germinated into the 'mixed ditype' outcomes which in parasexuality would be expected to be recovered with a higher frequency (ratio 2: 1, Fig. 1) than the total number of parental and non-parental ditypes (five germinated). The absence of mixed ditypes argues strongly that parasexual haploidisation is uncommon or not a part of the macrocyst cycle.

Table 2 Tetrad classification of individual macrocyst germinations in four crosses

Parental genotypes	Macrocyst tetrad classifications				Total no.
	PT	PD	NPD	TT	
(*plq*,+)×(+,*cac*)	7	2	1	6	16
(*plq*,+)×(+,*tag*)	12	0	0	5	17
(*cac*,+)×(+,*tag*)	11	1	0	4	16
(*cac*,*tag*)×wild type	22	0	1	2	25
Totals	52	3	2	17	74

Genotype and tetrad abbreviations are as in Table 1. The assignment of recombinant phenotypes was not difficult for any of the crosses. To prove the identification of the putative double mutant (*cac,tag*) it was back crossed to the wild type stock (line 4 above). Cactus and tag phentotypes were recovered in the single NPD and two tetratype macrocysts germinated. No unexpected progeny phenotypes were observed in any crosses, indicating that each mutant has a single lesion affecting its visible morphology.

An important question about the possible occurrence of multiple meioses is not totally resolved. The data in Table 2 are not consistent with the occurrence of independent karyogamies. In this case, few uniparental macrocysts should arise, and two kinds of expected tritypes ($aB+AB+ab$) and ($Ab+AB+ab$) were not found. Otherwise, mitoses of one original zygote before meiosis is still a possibility. This situation should never produce more than 50% ditypes from biparental macrocysts, whereas, a single meiotic event with no intrachromosomal crossing-over should yield 100% ditypes from the biparentals[8]. We are looking into this question, since any mutants which when crossed produce high frequencies of ditypes will also greatly facilitate the identification of linkage groups and mapping analyses.

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'Isotope effect' in metabolism of radioactive cortisol in man

TRITIUM-labelled steroids have been widely used as tracers in the investigation of steroid metabolism, due to their high specific activity, low cost and low radiation dosage *in vivo*. The validity of any experiment using labelled steroids depends on the fact that the labelled and the endogenous materials are metabolised in a similar manner. In the case of the *in vivo* metabolism of the 1,2-^{3}H-labelled and endogenous cortisol in man, proof of this is lacking. The experiments of Cope and Pearson[1], and of Gold and Crigler[2] using mixtures of 1,2-^{3}H-cortisol and 4-^{14}C-cortisol suggested that these two different species of labelled cortisol were metabolised in a similar manner, and from this it was inferred that they could both be used as valid tracers for studying cortisol metabolism.

In the course of a study of the uptake and metabolism of cortisol by the human forearm[3], we made the preliminary observation that following the intra-arterial injection of a mixture of 1,2-^{3}H-cortisol and 4-^{14}C-cortisol into two healthy men, cortisol isolated from both arterial and venous plasma from one subject had a lower isotope ratio (^{3}H : ^{14}C) than the injected mixture. We have now injected intravenously a mixture of 1,2-^{3}H-cortisol and 4-^{14}C-cortisol into six healthy men and have produced substantial evidence of an istope effect.

The two radioactive steroids were stated by the supplier to be at least 96% pure. Dilution analysis immediately before use showed that they were both at least 95% pure. Blood samples were obtained 2 min after the injection and at 30 min intervals thereafter for 4 h. Urine collections were made at 2, 4, 8, 12 and 24 h after the injection. Unlabelled cortisol (100 μg) and unlabelled cortisone (100 μg) were added to each plasma sample ($n=48$); corticosteroids were extracted using dichlormethane and separated by paper chromatography using the system benzene–methanol–water (2:1:1). The cortisol and cortisone fractions were further purified by paper chromatography using iso-octane–*t*-butanol–water (10:5:9) and on a thin layer of silica gel using chloroform–methanol–acetic acid (90:5:5). The isotope ratios were measured on the TLC eluates.

The principal findings are summarised in Table 1. Regression analysis shows a significant relationship (at the 0.1% level) between plasma cortisol isotope ratio and time. There were no significant differences between the isotope ratios of cortisol and cortisone.

After hydrolysis of aliquots of urine with β-glucoronidase, the tetrahydro metabolites of cortisol were extracted with ethyl acetate and separated by paper chromatography (benzene–methanol–water; 2:1:1). The eluted metabolites were treated with sodium borohydride and sodium metaperiodate and the resulting 11-hydroxy-17-oxosteroids were chromatographed on paper using toluene–iso-octane–methanol–water (80:120:160:40), and their isotope ratios determined. No significant differences were found in the ^{3}H : ^{14}C ratio of 3α,11β-dihydroxy-5β-androstan-17-one (11-HE) derived from 3α,17α,21-trihydroxy-5β-pregnan-11, 20-dione (THE) or from 3α,11β,17α,21-tetrahydroxy-5β-pregnan-20-one (THF), and that of the purified starting material (10.25 ± 0.35). However, in all 30 urine samples, 3α,11β-dihydroxy-5α-androstan-17-one (11-HA) derived from 3α,11β,17α,21-tetrahydroxy-5α-pregnan-20-one (5α THF) had a lower isotope ratio ($8.93 \pm 0.23, P < 0.001$) than 11-HE derived from either THE or THF, or from the starting material.

Table 1 Isotope ratio of labelled cortisol before and after purification, and after recovery from plasma (mean ± s.d.)

	^{3}H : ^{14}C
Material injected (n = 20)	10.00 ± 0.25
Material injected after purification as for plasma (n = 13)	10.25 ± 0.35*
Plasma 2 min after injection (n = 6)	9.95 ± 0.33
Plasma 4 h after injection (n = 6)	8.95 ± 0.19*

* Significantly different at the 0.1% level.

As the maximum degree of radiochemical impurity in each of the injected steroids was 5%, even the most unfavourable combination of circumstances could only account for less than half of the observed difference in isotope ratio between THE and THF, and 5αTHF. In the case of the plasma cortisol data, the fact that neither chromatographic purification nor a short period (2 min) *in vivo* results in a change in isotope ratio suggests that radiochemical impurities cannot account for the changes in plasma cortisol isotope ratio observed over a period of 4 h.

Gold and Crigler[2], and others, have shown that, in contrast to 4-^{14}C-cortisol, 1,2-^{3}H-cortisol tends to separate from unlabelled cortisol in simple chromatographic systems, a phenomenon that we also have encountered in the present study. These physiochemical findings support the view that the behaviour of 4-^{14}C-cortisol more closely resembles unlabelled cortisol than does the tritium-labelled form. The differences we have observed in the *in vivo* metabolism of the two labelled species may therefore indicate that there are similar differences between the metabolism of unlabelled cortisol and its 1,2-^{3}H-derivative.

While these findings do not necessarily invalidate the use of 1,2-^{3}H-cortisol for tracer studies there may be circumstances where knowledge of this effect could significantly influence the interpretation of results.

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The role of calcium ions in initiating transformation of lymphocytes

TRANSFORMATION of lymphocytes by antigens and by nonspecific mitogens (lectins and anti-immunoglobulin sera) is a consequence of the binding of the mitogen to specific receptors on the cell surface[1–3]. The receptors rapidly form clusters ('patches') which may aggregate into polar 'caps'[4] at higher mitogen concentration. These events initiate a series of biochemical changes—enhanced uptake of ions and metabolites[1], increased cyclic GMP[5], and increased metabolism of phosphatidyl inositol and other phospholipids (ref. 6 and V. C. M., M. J. Hayman and M. J. C., unpublished)—which culminate in DNA synthesis and mitosis 48–72 h later. Although the sequence, relative importance and control of these events is not yet defined, there is evidence for a primary role for Ca^{2+}. *Phaseolus vulgaris* phytohaemagglutinin (PHA) initiates a slow accumulation of Ca^{2+} by lymphocytes[7,8] and its stimulation of DNA synthesis is inhibited by citrate[9], EDTA (ref. 9) or EGTA (ref. 10). The inhibition is reversed by Ca^{2+}. It has also been shown that inhibition by the low concentrations (1.4 mM) of EGTA used did not prevent binding of PHA (ref. 11). Although these results showed Ca^{2+} to be an important factor in initiation of transformation it was not clear whether influx of Ca^{2+} was the only or even the main consequence of the binding of PHA.

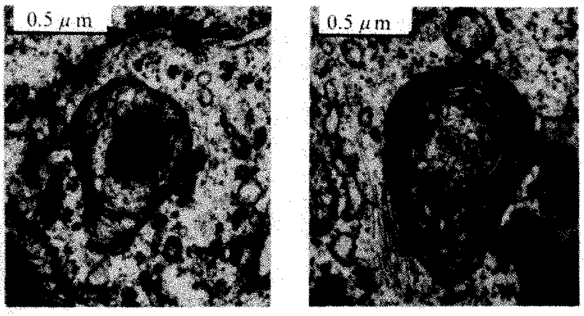

Fig. 1 Mitochondria from pig lymphocytes after 45 h in the presence of the ionophore A 23187 (0.8 μg ml^{-1}). Experimental details of the treatment are given under Fig. 2. In many cells up to one-third of the mitochondria showed dense granules, hypertrophied cristae or both. In dead cells the proportion of these forms was much higher.

Here we show that the Streptomyces antibiotic, A 23187, an ionophore specific for divalent cations[12], initiates a similar series of events to those caused by PHA. The ionophore is a monobasic carboxylic acid (molecular weight 531) two molecules of which form a lipid soluble complex with the cation and enable it to diffuse readily across biological membranes. Its structure has recently been determined[13]. We have studied its effect at micromolar concentration on the morphology of pig lymphocytes and on the incorporation of [6-^{3}H]-thymidine into DNA.

Pig lymphocytes were obtained from mesenteric lymph node as previously described[14]; about 15% of the cells were stained by a fluorescein-conjugated rabbit antiserum to pig immunoglobulin and on the basis of this criterion were judged to be B lymphocytes. Preliminary experiments showed a marked response to low concentrations of A 23187 (0.8 μg ml^{-1} per 10^6 cells). After 45 h culture the cells were indistinguishable under the light microscope from cells incubated with PHA and were agglutinated to a similar extent. Examination of electron micrographs revealed that 80–90% of the cells were enlarged (> 8 μm in diameter) and showed many of the features characteristic of PHA-transformed lymphocytes[15]. Thus, the cytoplasm was enlarged and showed marked endocytotic activity, the plasma membrane showed large pseudopodium-like extensions, there were many polysomes and an increased number of mitochondria. The main differences from PHA-treated cells were a lesser development of the rough endoplasmic reticulum and marked abnormalities of the mitochondria (Fig. 1) including large dense granules and hypertrophied cristae. In contrast with these enlarged cells, about 10% of the ionophore-treated cells appeared in all respects to resemble normal unstimulated lymphocytes and the possibility that A 23187 fails to activate a subpopulation of lymphocytes cannot be ruled out at present.

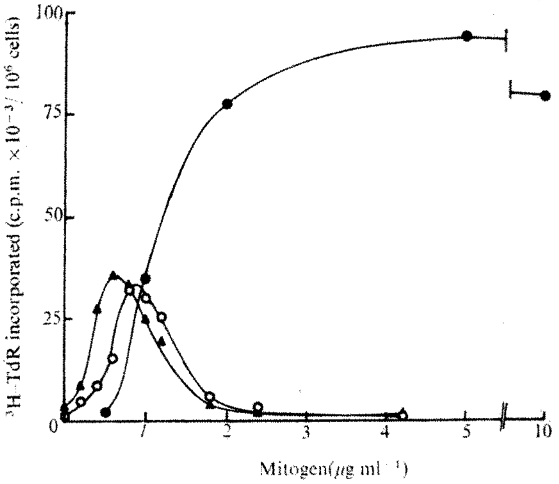

Fig. 2 Incorporation of ^{3}H-thymidine by pig lymphocytes as a function of concentration of A 23187 (○) or of PHA (●). The effect of sub-threshold amounts of PHA (0.3 μg, Wellcome Reagents Ltd) on the response to A 23187 is also shown (▲). The assays were performed as described previously[14], using cells (10^6) from pig mesenteric lymph node in 1 ml of Eagle's medium (containing 1.8 mM $CaCl_2$, 1 mM $MgCl_2$) supplemented with 20% (v/v) foetal calf serum. A 23187 was dissolved in ethanol (1.5 mg ml^{-1}) and diluted with 0.5 volumes of 10 mM Tris HCl buffer, *p*H 7.4; solutions were stored at 2° C in the absence of light and were used up to 4 weeks after preparation. Cultures were incubated at 37° C in an atmosphere of air containing 5% (v/v) CO_2 for 45 h before the addition of ^{3}H-thymidine (1 μCi) and for a further 5 h before recovery of the DNA. The reported values represent the means of duplicate estimations. The PHA curve was obtained with a different batch of cells from that used for the other two curves. The cells used for the A 23187 gave an incorporation of 140,000 c.p.m. with 2 μg PHA (see Fig. 5).

To obtain a quantitative measure of the stimulus, we followed the uptake of ^{3}H-thymidine into DNA after exposure to A 23187 for 45 h. The response to increasing amounts of ionophore is compared with that to PHA in Fig 2. Both PHA and A 23187 gave a sharp increase in thymidine uptake when their concentrations exceeded a certain critical threshold value and maximal incorporations of thymidine were induced by 0.8–1.0 μg of A 23187 and 2–4 μg of PHA. The response to PHA fell relatively slowly at higher concentrations whereas that to A 23187 declined sharply when the optimal concentration was exceeded. The absolute amounts of radioactivity varied considerably from one batch of cells to another but the maximal stimulation by the ionophore was usually about one-third to one-quarter of that caused by PHA. In view of this morphological evidence in support of transformation, it seems likely that the lower thymidine incorporation induced by A 23187

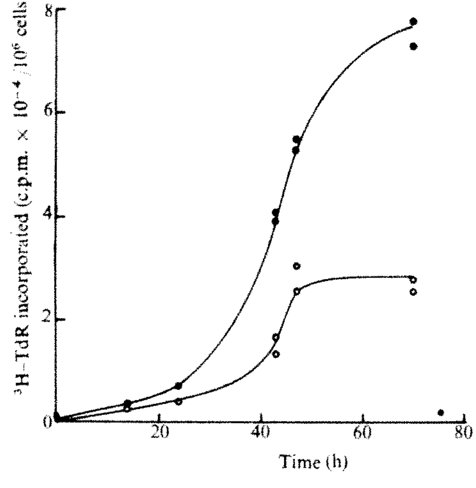

Fig. 3 Time course of ^{3}H-thymidine incorporation by pig lymphocytes in the presence of A 23187 (○) and PHA (●). Assays were performed as previously described using amounts of A 23187 (0.8 μg ml^{-1}) and PHA (2 μg ml^{-1}) giving maximal stimulation. ^{3}H-thymidine was incubated with cultures for a period of 4 h before the cultures were removed and the DNA was recovered.

relative to PHA does not represent a lower proportion of activated cells but is due to the perturbation of the metabolism of the cell by the penetration of the ionophore to the mitochondria.

We have not yet checked whether all the biochemical effects of PHA are duplicated by A 23187, but the parallelism of the morphological responses suggests a similar biochemical basis. Furthermore, the experiment shown in Fig. 3 demonstrates that thymidine uptake follows a similar time course with the two different stimuli.

If both mitogens act by facilitating entry of Ca^{2+} then it should be possible to obtain a response by combining subthreshold amounts of each. The curve (▲) in Fig. 2 shows this effect when the response to A 23187 was measured in the presence of 0.3 μg ml^{-1} PHA, which by itself had little effect (^3H-TdR incorporated: 3,600 c.p.m. 10^6 cells). This result also suggests that largely overlapping populations of cells were involved in the response to both agents. The converse experiment showing PHA response in the presence of A 23187 was complicated by inhibitory effects of the ionophore which will be considered later.

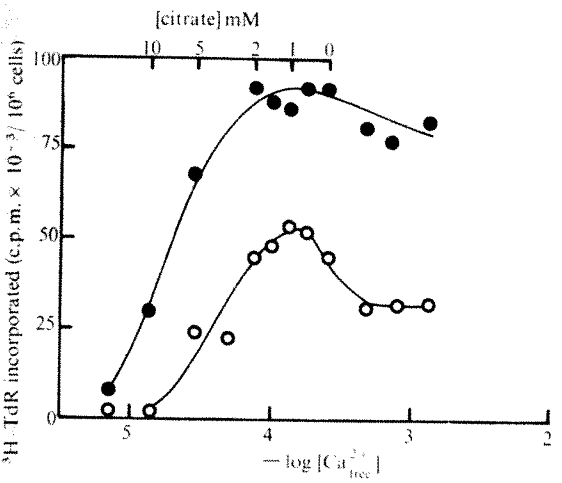

Fig. 4 Effect of concentration of Ca^{2+} on response of lymphocytes to PHA (●) or A 23187 (○). The experiments were carried out in foetal calf serum ($[Ca^{2+}]=3.2$ mM, measured spectrophotometrically using murexide[16]) diluted to 1:10 with Ca^{2+} free Eagle's medium. Ca^{2+} concentration was controlled by addition of Ca^{2+} or sodium citrate. In those samples in which extra Ca^{2+} was added it was assumed that 60% was free, the remainder being bound to PO_4 (1 mM, $K=1.8$ mM) or serum albumin (0.2 mM in Ca^{2+} binding sites, $K=1$ mM[17]). In those samples in which Ca^{2+} was complexed by citrate the concentration of bound divalent cation (x) was calculated from the total $[Ca^{2+}+Mg^{2+}]$ (M) and the concentration of citrate (C) and the dissociation constant $K=$ 0.5 mM, which is almost the same for both ions[18]. $2x=(M+K+C)-[(M+K+C)^2-4MC]^{1/2}$. The fraction of free $Ca^{2+}=(1-(x/M))$.

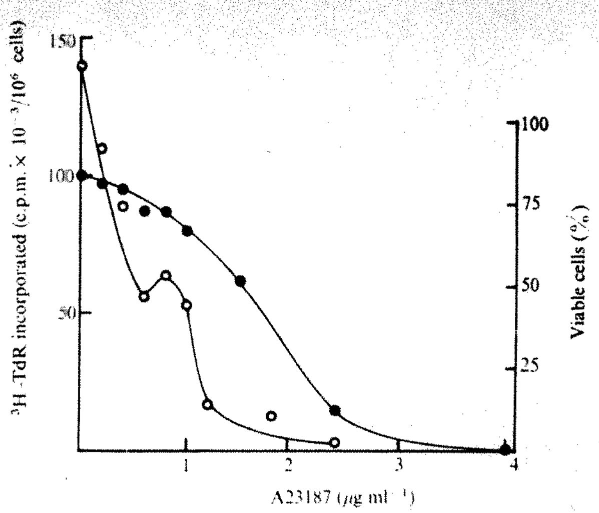

Fig. 5 Effect of A 23187 on incorporation of ^3H-thymidine into pig lymphocytes stimulated by 2 μg PHA per 10^6 cells (○) and the viability of the cells (●). The experimental procedure is given under Fig. 1. PHA and A 23187 were both added at the beginning of the experiment. The viability after 45 h of incubation with A 23187 was measured as the percentage of cells which excluded trypan blue.

The critical role of Ca^{2+} in both responses was confirmed by lowering its concentration in the medium (Fig. 4). First, a basic Ca^{2+} free medium was supplemented with 10% (v/v) of foetal calf serum, instead of the usual level of 20%, to give a Ca^{2+} concentration of 0.3 mM. This lowering of Ca^{2+} barely affected the response to PHA but it significantly enhanced the response to the ionophore. Further lowering of Ca^{2+} was effected by addition of sodium citrate at concentrations between 1 mM and 10 mM. The results in Fig. 4 show that there was a sharp drop in response to A 23187 when $[Ca^{2+}]$ fell below 10^{-4} M, close to the dissociation constant of the complex between Ca^{2+} and A 23187[19]. The response to PHA fell when a slightly lower Ca^{2+} concentration was reached (half maximal response at 2×10^{-5} M) in good agreement with the results of Alford[9] and of Whitney and Sutherland[10]. We conclude that A 23187, like PHA, requires Ca^{2+} in the external medium in order to be effective. Addition of a 1 mM excess of Ca^{2+} over citrate (5 mM and 10 mM) restored the response both to PHA and to A 23187 to the level of the controls.

The inhibitory effects of A 23187 on the response to PHA are shown in Fig. 5, together with a curve showing the decrease in viability of the cells. Although the two curves show a rough correspondence it is clear that there is initially a marked inhibition without significant loss of viability and that later, above the normal threshold for stimulus by A 23187, an activating effect is superimposed on the declining incorporation of thymidine. It is not easy to explain this activation if a single population of cells, already fully stimulated by the PHA, is involved, unless A 23187 has some effect in addition to facilitating the influx of Ca^{2+}. Another explanation is that A 23187 can stimulate a subpopulation of lymphocytes which are not affected by the PHA. This would be consistent with the high proportion of cells which enlarge under the influence of the ionophore. Experiments to distinguish between these possibilities are in progress.

It is likely that much of the toxic effect of the ionophore can be explained in terms of its effects on the mitochondria, the morphology of which is grossly disturbed. It is well known that mitochondria have a large capacity for taking up Ca^{2+}, especially in the presence of phosphate and other permeant anions[20] and it is also known that A 23187 stimulates this uptake and diverts the energy from both respiration and from exogenous ATP into Ca^{2+} transport[16]. As inhibitors of glycolysis and of respiration are known to block transformation[21] it is not surprising that A 23187 is also inhibitory. Mouse and rat spleen cells were more sensitive than pig lymphocytes to the toxic effects of the ionophore, only 20% surviving after 40 h at 0.8 μg ml^{-1}, and we have been unable to produce significant stimulation of thymidine uptake by these cells.

Except for its inhibitory effects, A 23187 produces a very similar response in pig lymphocytes to that produced by PHA. In addition to the results described above we have preliminary evidence which shows a stimulus of phosphatidyl inositol turnover and an inhibition of PHA stimulated phosphatidyl inositol turnover by citrate. If the effect of the ionophore is limited to its facilitation of divalent cation movements then those of PHA can be similarly explained We therefore suggest that lymphocyte transformation is the result of a direct effect of the mitogen on the permeability of the plasma membrane to Ca^{2+}

Two interesting questions follow First, how does PHA increase permeability to Ca^{2+} and, second, what are the further effects of Ca^{2+} inside the cell?

The most striking common feature of the action of a variety of mitogens on lymphocytes is the requirement for multivalency[1 3] This generalisation also applies to the release of histamine from mast cells by antibodies to IgE, a response which can also be induced by A 23187 in the presence of Ca^{2+} (ref 22) If the receptor proteins bridge the lipid regions of the membrane then it is plausible to suggest that clusters of receptors might contain polar channels permitting the passage of Ca^{2+} and possibly of other ions into the cell This may be but one example of a general mechanism for transmitting signals across a membrane following the interaction between a specific receptor and the appropriate stimulus

Many intracellular processes are controlled by the concentration of Ca^{2+} (ref 23), at least three of these are involved before DNA synthesis in stimulated lymphocytes Activation of phosphorylase kinase[24] is required for the initiation of glycolysis[21], the microfilament–microtubule system is required for endocytosis[25] and its inactivation blocks transformation[26], lastly and most significantly, the level of cyclic GMP in some cells has been shown to be controlled by Ca^{+2} (ref 27) and its concentration in lymphocytes rises immediately in response to PHA (ref 5) The further effects of cyclic GMP are not yet defined but in other tissues it has been implicated in several hormonal responses[28] and it is clearly an important intermediary

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Actions and interactions of colchicine and cytochalasin B on contraction of granulation tissue and on mitosis

It has been shown by Majno *et al*[1] that a strip of growing granulation tissue (GT) cut from the wall of a 2- to 4-week-old granuloma pouch[2] or from the base of an open wound in the rat, contracts *in vitro* in response to 10^{7}–10^{6} concentrations of 5-hydroxytryptamine, angiotensin and other agonists An association between this pharmacological reactivity of GT and the phenomenon of wound contraction has been proposed[1] on the bases that the forces which cause a wound to contract are cellular in origin and generated by GT cells[3,4], and that fibroblasts proliferating *in vitro* and *in vivo* develop a muscle-like cytoarchitecture characterised by an increase in the number of microfilaments[5] Also fibroblasts in culture contain abundant microtubules[6] which can be rapidly destroyed by poisons such as colchicine[6–8] that prevent polymerisation of the tubular protein For example colchicine can prevent microtubules comprising the mitotic spindle from forming and thereby inhibit karyokinesis (separation of chromosomes)[9] The drug seems not to affect the functions of microfilaments, on which various types of contractile activity, including cytokinesis, depend[10], the microfilaments are specifically inhibited by the drug cytochalasin B (ref 11)

We performed a series of experiments using the technique of Majno *et al*[1], primarily to determine the time of onset of contractility of GT after wounding and the effects of X irradiation on contractility We found that when colchicine or other spindle poisons (vincristine, vinblastine or podophyllotoxin) were added to GT in the bath in 10^{-7}–10^{-6} final concentrations, there slowly developed a marked contraction of the tissue, which was shortened by as much as 30–40% of its initial length (Fig 1*a*) Contraction induced by colchicine was dependent on the presence of oxygen and was inhibited by 10^{-4}–10^{-5} 2, 4-dinitrophenol (DNP) (Fig 1*b*) Contraction of GT induced by colchicine and related drugs was also reduced by 10^{-5} KCN and by 10^{-4} papaverine After contraction of GT had been induced by colchicine, the tissue failed to relax after five or more successive washes with fresh Tyrode solution, which did not free the tissue of the drug ATP caused GT to contract and DNP caused relaxation Colchicine failed to cause contraction of GT in the presence of DNP, when the tissue was washed, DNP but not colchicine was removed and the tissue recontracted at the same rate as before the addition of DNP (Fig 1*b*) It was more difficult to reverse the action of papaverine on contraction caused by colchicine by washing in this way

When 10^{-7}–10^{-6} cytochalasin B (dissolved in DMSO[11]) was added to GT, the tissue relaxed slightly, subsequent washing restored the initial base line and the preparation then contracted in the usual way on addition of colchicine When 10^{-6} cytochalasin B was added to the bath, followed (without washing) by 10^{-6} colchicine, the tissue failed to contract, until it was washed with fresh Tyrode solution, this removed cytochalasin B as it removed DNP, and allowed contraction to occur When cytochalasin B was added after contraction had been induced by colchicine, the tissue relaxed completely (Fig 1*d*), further washing caused contraction to re-occur, at the same rate as prior to the addition of cytochalasin B in the same way as shown for DNP (Fig 1*b*)

This reactivity of GT to spindle poisons, metabolically dependent on the presence of oxygen and on oxygen-coupled phosphorylation, and antagonised by cytochalasin B, was shown by strips of GT cut from 7–35-d-old pouches in the rat We have been unable to demonstrate any pharmacodynamic activities of strips of uninjured subcutaneous connective tissue removed from rats by adding colchicine, cytochalasin B, 5-hydroxytryptamine, angiotensin, histamine or other agonists But colchicine caused oxygen-dependent contractions, also inhibited by DNP and cytochalasin B, of rat stomach fundus and of rat and guinea pig ileum, similar to GT

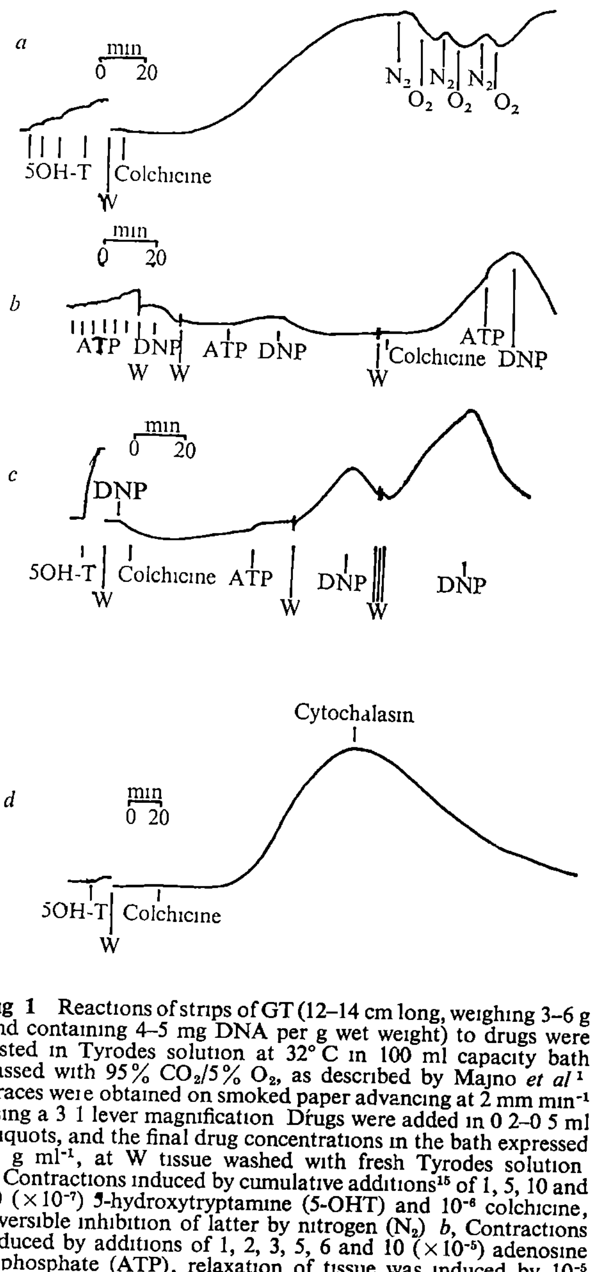

Fig 1 Reactions of strips of GT (12–14 cm long, weighing 3–6 g and containing 4–5 mg DNA per g wet weight) to drugs were tested in Tyrodes solution at 32° C in 100 ml capacity bath gassed with 95% CO_2/5% O_2, as described by Majno *et al*[1] Traces were obtained on smoked paper advancing at 2 mm min^{-1} using a 3 1 lever magnification Drugs were added in 0 2–0 5 ml aliquots, and the final drug concentrations in the bath expressed as g ml^{-1}, at W tissue washed with fresh Tyrodes solution *a*, Contractions induced by cumulative additions[15] of 1, 5, 10 and 50 ($\times 10^{-7}$) 5-hydroxytryptamine (5-OHT) and 10^{-6} colchicine, reversible inhibition of latter by nitrogen (N_2) *b*, Contractions induced by additions of 1, 2, 3, 5, 6 and 10 ($\times 10^{-5}$) adenosine triphosphate (ATP), relaxation of tissue was induced by 10^{-5} 2,4-dinitrophenol (DNP) which also inhibited the contraction due to 10^{-6} colchicine *c*, Shows GT failed to contract to 10^{-6} colchicine in the presence of 10^{-5} DNP until the tissue was washed with fresh medium which removed DNP and caused recontraction due to colchicine *d*, Contractions induced by 10^{-6} colchicine inhibited by 10^{-5} cytochalasin B (DMSO used as solvent for cytochalasin B had no effect on contraction of GT)

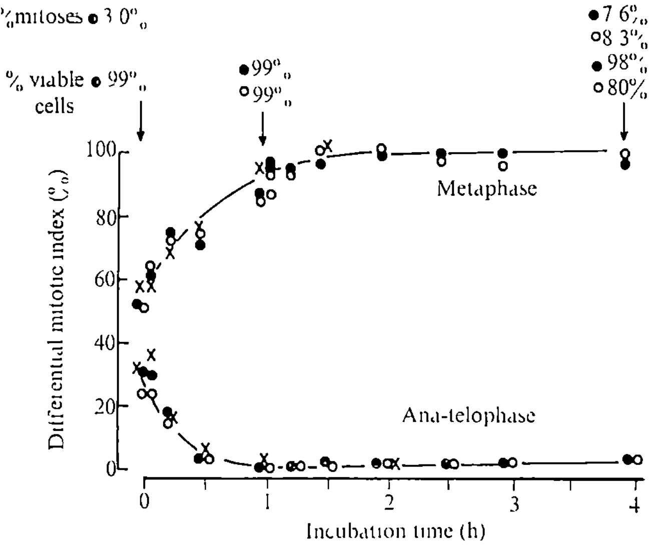

Fig 2 Metaphase arrest induced by 10^{-6} colchicine in freshly collected W-256 cells (~ 10^6 cells ml^{-1}) incubated at 36° C in medium 199 plus 10% horse serum and gassed with 95% O_2/5% CO_2 (●) or N_2 containing < 3 p p m O_2 (○) The presence of 10^{-5} cytochalasin B failed to influence metaphase arrest induced by 10^{-5} colchicine under aerobic conditions (×) Percentage viable cells determined using nigrosin exclusion technique, total mitotic counts based on all mitotic stages present in 500–1,000 cells per sample, differential mitotic counts based on 100 metaphases counted in each aliquot removed after 0–4 h incubation time

We measured the effect of colchicine and cytochalasin B under aerobic and anaerobic conditions on mitotic division of W-256 rat tumour cells cultured *in vitro*, which had been collected from ascites fluid when the tumour was at an exponential phase of growth Differential mitotic counts were made on cell aliquots removed from the cultures at intervals after the drug(s) had been added Two telophase stages were scored cytoplasmic cleavage absent (1), and present (2) to establish cytokinesis During incubation, the cells were bubbled with either 95% O_2/5% CO_2 or with pure N_2 (containing <3 p p m O_2) Colchicine caused metaphase arrest irrespective of the presence or absence of oxygen or cytochalasin B (Fig 2, Table 1) But 10^{-5}–10^{-4} DNP, greatly reduced the effect of colchicine on cell division Cytochalasin B inhibited cytokinesis Neither the absence of oxygen nor the presence of papaverine affected karyokinesis and cytokinesis significantly, but cytochalasin B was significantly less effective in preventing cytokinesis when oxygen was absent or DNP present (Table 1)

Assuming that colchicine and cytochalasin B act specifically on microtubules and microfilaments respectively, the results obtained indicate that both organelles participate in the maintenance of 'tone' of the contractile cells which develop in GT and are present in stomach and ileum It is suggested that in cell contraction a microtubular apparatus functions primarily by regulating the tone of the contractile component (microfilaments), that the destruction of microtubules by colchicine releases the microfilaments from the control exercised by the microtubules and indirectly causes contracture to develop, provided oxygen is present and coupled to phosphorylation, and that cytochalasin B inhibits contracture by a specific direct action on microfilaments

The intracellular movements which take place during mitosis are attributed to successive changes occurring in microtubules (karyokinesis) followed by microfilaments (cytokinesis) The results obtained indicate that neither karyokinesis

Table 1 Differential counts of mitoses in W-256 cells incubated for 100 min in presence of colchicine and other compounds under aerobic or anaerobic conditions

Final concentration of compound (g ml^{-1})	Gas phase	Total mitoses	Differential mitotic count* % P	M	A	T 1	T 2	Significance†
Nil	Oxygen	247	13	40	7	10	30	
Nil	Nitrogen	188	9	52	4	7	28	
10^{-7} colchicine	Oxygen	120	8	84	2	1	5	
10^{-6} colchicine	Oxygen	118	8	85	1	0	6	
10^{-6} colchicine	Nitrogen	113	3	89	2	2	4	
2 × 10^{-5} cytochalasin B	Oxygen	255	9	39	7	43	2	a $P < 0.01$
2 × 10^{-5} cytochalasin B	Nitrogen	184	2	54	10	24	10	
2 × 10^{-5} cytochalasin B plus 10^{-6} colchicine	Oxygen	106	4	94	1	1	0	
10^{-4} papaverine	Oxygen	216	8	47	4	6	35	
Nil	Oxygen	207	14	49	5	8	24	
10^{-5} 2, 4 DNP	Oxygen	217	9	46	4	5	36	
10^{-4} DNP	Oxygen	177	18	57	1	3	21	
10^{-5} 2, 4 DNP plus 10^{-7} colchicine	Oxygen	193	9	60	3	5	23	
10^{-4} 2, 4 DNP plus 10^{-6} colchicine	Oxygen	161	7	63	2	3	25	b $P < 0.001$
10^{-6} colchicine	Oxygen	113	6	90	0	1	3	
2 × 10^{-5} cytochalasin B	Oxygen	198	11	51	5	30	3	c $P < 0.01$
10^{-4} 2, 4 DNP plus 2 × 10^{-5} cytochalasin B	Oxygen	219	11	46	2	25	16	

Freshly collected W-256 cells in log growth phase were incubated in medium 199 (plus 10% horse serum) at 36° C. Approximately 10^6 cells were added per ml of medium which had been prewarmed and gassed for 20 min with 95% O_2/5% CO_2 or N_2 before and after addition of the various compounds followed by cells. After incubation, the cells were fixed by adding an excess of methanol–acetic acid (3:1 v/v) and were then stained with acetic orcein. Differential mitotic counts are based on 100 metaphases counted in each preparation.

*Abbreviations: P, prometaphase, M, metaphase, A, anaphase, T, telophase (cytoplasmic cleavage absent (1) or present (2)).

†Based on χ^2 test for T(2)/(A + T1 + T2) in *a* and *c* and for (A + T1 + T2) in *b*.

nor inhibition of spindle synthesis by colchicine require the presence of significant concentrations of oxygen, the considerable energy required for mitosis seems to be supplied from reservoirs built up progressively during the whole cell cycle[12]. It has been found that a slight increase in oxygen consumption occurs at cytoplasmic cleavage of sea urchin eggs[12,13]. We found that anoxia maintained for 100 min caused a 12% decrease in the proportion of type 2 telophase in W-256 cells but this decrease was not statistically significant. DNP caused no significant delays in the progression of the cells through mitosis, but inhibited the actions of colchicine on karyokinesis and of cytochalasin B on cytokinesis. This suggests that uncoupling of phosphorylation by DNP affects active transport of colchicine or possibly its binding to the target protein (microtubule) of the dividing cell. This binding is, however, reversible and does not involve a chemical modification of colchicine[7], it also possibly affects transport and binding of cytochalasin to the contractile microfilaments. Ultrastructural studies have provided some evidence that microtubule subunits may aggregate to form 100 Å filaments[14].

A comparison of the effects of colchicine, cytochalasin, DNP and oxygen on cell division and on contraction of GT shows that these two cellular activities react similarly. Our findings suggest that microtubules are closely integrated with microfilaments in regulating both these processes. The termation of mitosis in telophase may reflect a loss of control of the tubular component over the microfilaments which causes a spontaneous contracture of the latter and results in cell cleavage, in the same way that inactivation of the corresponding tubular component (by colchicine) in the contractile cell produced contracture. Contracture induced by colchicine, and other spindle poisons, was all-or-none in character, it was not possible to regulate the degree of tissue shortening by altering the drug concentration. This is the type of response which would be caused by a drug which destroys the mechanism on which it acts. Cell cleavage appears to be a phenomenon with similar all-or-none characteristics.

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Reversibility of morphine tolerance and dependence in guinea pig brain and myenteric plexus

WHEN guinea pigs are exposed continuously to high morphine concentrations, tolerance and physical dependence develop within a few days in both the central nervous system (CNS) and the myenteric plexus. In the plexus morphine tolerance is associated with subsensitivity to the inhibitory catecholamines and supersensitivity to the excitatory neurotransmitter serotonin (5-hydroxytryptamine, 5-HT)[1,2]. We have investigated the following questions about these phenomena. (*a*) After opiate withdrawal in animals made tolerant and dependent, would the altered sensitivities to neurotransmitters in the plexus return to normal? (*b*) If so, would loss of tolerance to morphine in the plexus proceed in parallel with loss of subsensitivity to catecholamines and loss of supersensitivity to 5-HT? (*c*) Would such reversion to normal in the plexus be concurrent with disappearance of CNS indications of tolerance and dependence?

Male guinea pigs (400–450 g) were rendered tolerant and dependent in 3 d by implanting four morphine pellets (75 mg morphine base each) subcutaneously[3] The pellet residues were removed on the third day after implantation and the animals were maintained for 7 d thereafter Implantation and removal were carried out under light ether anaesthesia

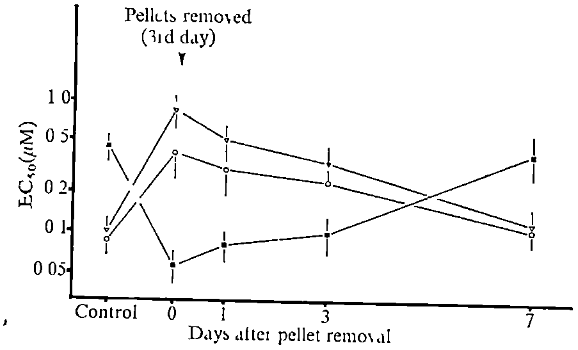

Fig. 1 Changes in sensitivity to morphine (▽), adrenaline (○), and 5-HT (■) of the longitudinal muscle-myenteric plexus preparation of guinea pigs the third day after implantation with four morphine pellets (75 mg morphine base each) and after abrupt withdrawal by pellet removal (third day after implantation) The mean effective dose (EC_{50})± s e m of each drug is plotted against time on a logarithmic scale Each point is based on four to five animals (eight to ten strips)

The longitudinal muscle strip with attached myenteric plexus from the ileum was prepared according to Rang[4] and mounted in a 5-ml tissue bath as described previously[1] The bath contained Krebs bicarbonate Ringer solution (37° C) and was bubbled with 95% O_2/5% CO_2 Electrical field stimulation for maximal twitch tension was applied at 80 V, 0 1 Hz and 0 5 ms pulse duration by means of a Grass S4E stimulator Strips were equilibrated for 1 h with electrical stimulation and washed at intervals of 5–10 min A force displacement transducer (Grass FT 0 03C) connected to a Grass 7B polygraph recorded the isometric twitches (2–4 g tension) Resting tension was adjusted to 1 g

Longitudinal muscle strips of untreated animals were prepared after overnight starvation Morphine-treated animals were killed, and strips were obtained on the third day after pellet implantation, and on the first, third and seventh day after pellet removal When morphine or catecholamines were tested, electrical stimulation was applied continuously The drug was washed out after a maximal effect was established Excitatory compounds, like acetylcholine and 5-HT, were added to the bath 10 s after stopping electrical stimulation Drugs were added to the bath at intervals of 10–45 min The concentration required for morphine or catecholamines to inhibit electrically induced twitches by 50% is called EC_{50} The same term is used for induction of 50% of the preceding electrically stimulated tension by acetylcholine or 5-HT

Tolerance in guinea pigs was measured by the effect of intravenously injected morphine sulphate (50 mg free base ml^{-1}) on the body temperature In controls, 30 min after intravenous injection of 10 mg kg^{-1} morphine, a maximal degree of hypothermia was observed, using a thermistor probe inserted 5 cm into the rectum for 2 min Physical dependence was tested by precipitation of jumping behaviour on intraperitoneal injection of naloxone hydrochloride (50 mg ml^{-1}), as previously described for guinea pigs[1] Each animal was tested only once

Figure 1 summarises the responses to morphine, adrenaline, and 5-HT of longitudinal muscle–myenteric plexus preparations isolated from guinea pigs during and after exposure to morphine After removal of the pellets, tolerance to morphine was gradually lost, as indicated by the decrease of the effective concentration (EC_{50}) required for 50% inhibition of electrically induced twitches Within 7 d the tissue had recovered normal sensitivity to the opiate (EC_{50}=100 nM)

A similar time course of recovery was found for adrenaline, with virtually normal sensitivity restored by the seventh day after pellet removal

Sensitivity to the excitatory effect of 5-HT followed a very similar but inverse course Supersensitivity reached about eight-fold (EC_{50}=55 nM) compared with the non-tolerant preparations (EC_{50}=420 nM) The first day after pellet removal the strips were still six times more sensitive to 5-HT than controls, and even on the third day some supersensitivity persisted (EC_{50}=100 nM) As described for the other drugs, normal sensitivity was established by the seventh day (EC_{50}=400 nM)

The dose-response curve for dopamine inhibition in morphine tolerant strips was very flat, with only 20% inhibition at 100 μM (Fig 2) The same results were obtained the first day after pellet removal, and even after 3 d, 100 μM dopamine only depressed the twitch tension 40% Nevertheless, normal sensitivity had returned within 7 d (EC_{50}=10 μM)

The drugs used in the above experiments produce a common effect, they influence the output of acetylcholine induced by electrical stimulation or by 5-HT Since acetylcholine added to the bath stimulates the longitudinal muscle directly[5], the response to this neurotransmitter may indicate whether or not chronic exposure to morphine affects the sensitivity of the muscle We noticed no change in sensitivity to the excitatory effect of acetylcholine in strips prepared before or after pellet removal as compared with controls (EC_{50}=0 4 nM) Furthermore, no change in twitch tension induced by maximal electrical stimulation was seen at any time in these experiments

To determine tolerance in the CNS, the hypothermic action of morphine was measured 30 min after intravenous injection A dose of 10 mg kg^{-1} caused a fall of rectal temperature of 0 8° C in controls (Table 1) The same dose,

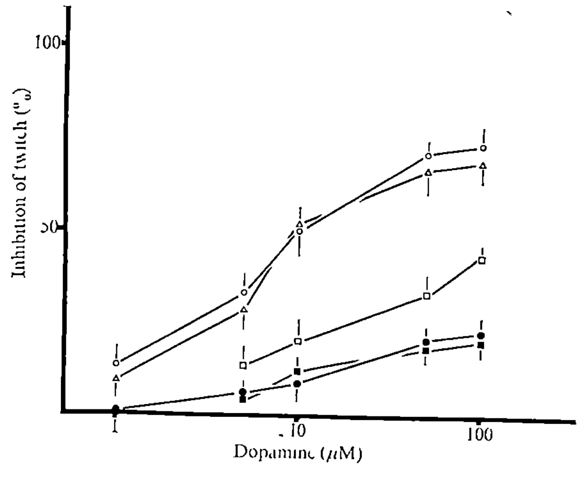

Fig 2 Dose-response curves for dopamine inhibition of the electrically stimulated muscle twitch Control (○) strips, preparations from guinea pigs 3 d after pellet implantation (■), and the first (●), third (□), and seventh (△) day after pellet removal Each point represents the mean depression (±s e m) of the electrically stimulated twitch (N=4–5) For further explanation, see Fig 1

3 d after pellet implantation, depressed the temperature by only 0.3° C. After pellet removal, there was no change in the degree of tolerance after 1 d, partial recovery by the third day and complete recovery by the seventh day.

Physical dependence disappeared at approximately the same rate as tolerance to hypothermic effects. As Table 1 shows, after pellet removal naloxone became gradually less effective in precipitating withdrawal jumping; and after 7 d, no jumping at all was observed, even with doses up to 128 mg kg^{-1}.

The results reported here demonstrate that pharmacological alterations observed during morphine tolerance and dependence in the guinea pig myenteric plexus as well as in the CNS are completely reversible. Within 7 d following abrupt withdrawal, sensitivities to morphine, naloxone, adrenaline, dopamine and 5-HT returned to normal. Thus, these findings support the view that opiate tolerance and dependence can disappear rapidly and completely[6-10]. By the methods used here we were unable to observe any persistence of tolerance or dependence[11-14], or of the associated abnormal sensitivities to neurotransmitters.

Table 1 Effect of morphine pellet implantation and removal on tolerance and physical dependence in guinea pigs

	Temperature fall*			Naloxone ED_{50}‡
		N	*P*†	(mg kg^{-1} ± s.e.m.)
Control	−0.80 ± 0.14	5	—	> 100¶
Third day after pellet implantation	−0.30 ± 0.05	6	< 0.01	2.4 ± 0.9
First day after pellet removal§	−0.32 ± 0.13	6	< 0.01	3.2 ± 1.2
Third day after pellet removal	−0.48 ± 0.07	5	< 0.01	41.0 ± 3.7
Seventh day after pellet removal	−0.89 ± 0.14	5	N.S.	> 128¶

Data show the effect of morphine (10 mg kg^{-1} intravenous) on the rectal temperature and the ED_{50} of naloxone hydrochloride (intraperitoneal) required to precipitate withdrawal jumping.

*Mean °C ± s.e.m., measured at room temperature 20°C.

†Two-tailed *t* test for difference from control.

‡The median effective dose (ED_{50}) ± s.e.m. consists of three to four independently calculated ED_{50} values according to Dixon[18]. Each independent ED_{50} is based on three to four animals.

§Pellet removal on third day after implantation.

¶This dose did not evoke jumping.

Our data show that the time course of recovery after morphine withdrawal is very similar in the myenteric plexus and CNS. This parallelism supports the likelihood of a common underlying mechanism in both nervous tissues. Furthermore, the results obtained in the plexus are in accord with our earlier findings that morphine tolerance is accompanied by supersensitivity to the excitatory neurotransmitter 5-HT[1,2], as first hypothesised by Collier[15]. As discussed[16], this supersensitivity could be a sufficient condition for both opiate tolerance and dependence. The supersensitivity is seen as an adaptive response to chronic inhibition by morphine of nerve pathways containing at least one serotoninergic synapse. Then more morphine would be required to inhibit (that is, tolerance), and a certain amount of morphine would be required to normalise the supersensitive synapses (that is, dependence). When exposure to morphine ends and the ambient morphine concentration declines, the disinhibition of these synapses could account for the abstinence syndrome. In support of the hypothesis is the finding that cyproheptadine, a specific 5-HT receptor blocker in the CNS, abolishes most withdrawal phenomena[17]. The time course of the loss of supersensitivity to 5-HT after morphine withdrawal, which corresponds to the rate of disappearance of tolerance and dependence in the CNS, may reflect the turnover rate of 5-HT receptors.

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Isolation of photoreceptor and conventional nerve terminals by subcellular fractionation of rabbit retina

LITTLE is known of retinal transmitter substances although acetylcholine, dopamine, 5-HT, γ-aminobutyric acid (GABA), glutamate, and aspartate have all been put forward as candidates for such a role[1-9]. In other areas of the central nervous system (CNS), the preparation of isolated nerve-ending particles (synaptosomes) has been extremely valuable in studying central transmitter substances[10-13]. As far as we are aware, however, subcellular fractionation procedures have not previously been applied to the study of retinal transmitter substances.

Here we have subjected the rabbit retina to homogenisation and subcellular fractionation[12,14] and have found that these procedures produce synaptosomes, not only from the conventional type of retinal nerve ending, but also from easily identifiable pinched-off photoreceptor terminals. We have also studied the subcellular distribution in the retina of some endogenous amino acids and of some marker enzymes, particularly those concerned with GABA metabolism. The distribution of exogenously accumulated ^{14}C-GABA and ^{3}H-dopamine was also examined[15]. The results of these experiments support the suggestion that aspartate may be a photoreceptor transmitter and that GABA and dopamine may be transmitters at some of the conventional retinal synapses.

Rabbit retinae were homogenised in 0.32 M sucrose and a crude nuclear pellet (P1) and a crude mitochondrial

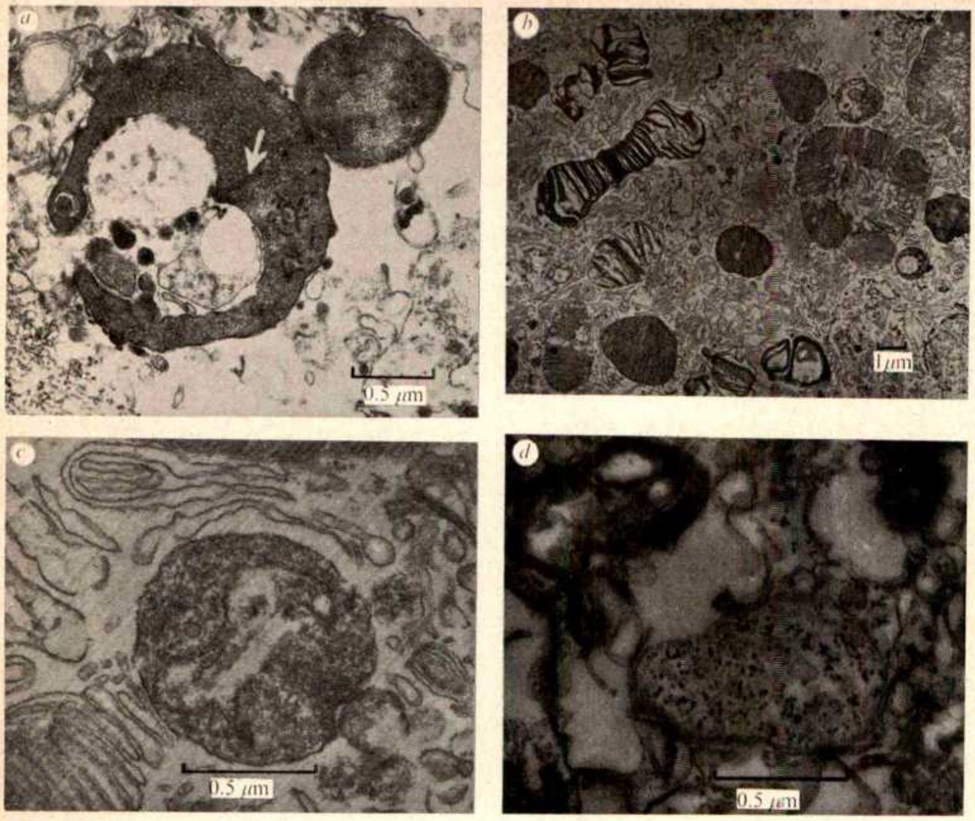

Fig. 1 Electron micrographs of retinal subcellular particles. *a*, Rod photoreceptor terminal: The arrow points to the synaptic ribbon, paired invaginations lie one on each side of the ribbon. Between the ribbon and the invaginations is the arciform density. A sheet of synaptic vesicles lie apposed to the ribbon. *b*, Fragments of photoreceptor outer segment; *c*, synaptosome from a conventional retinal synapse, *d*, particle possibly originating from glia and containing many small electron opaque granules but no synaptic thickening.

pellet (P2) (ref. 12) were obtained by differential centrifugation. For electron microscopy the P1 and P2 pellets were fixed in glutaraldehyde, post-fixed in osmium tetroxide and embedded in Epon–araldite. Sections were stained with methanolic uranyl acetate and aqueous lead citrate and examined by electron microscopy.

The crude nuclear fraction (P1) contained many nuclei, tissue fragments, and a few large mitochondria. Aggregations of smaller mitochondria were frequently seen and these appeared to be derived from sections of photoreceptor inner segments being sheared-off intact during the homogenisation process. In addition, fragmented outer segments were frequently observed. The most striking feature of this fraction, however, was the presence of large numbers of pinched-off rod photoreceptor terminals (Fig. 1*a*). These structures were identified by the presence of densely staining cytoplasm, many synaptic vesicles and a synaptic ribbon with an associated halo of synaptic vesicles. Most of these rod photoreceptor terminals, (diameter 1–2 μm) were sectioned in a plane which revealed the invaginations into which processes from horizontal and bipolar cells normally project[16,17]. Occasionally a much larger cone terminal (diameter 5–10 μm) with less dense cytoplasm was observed. A few nerve endings with light staining cytoplasm (diameter approximately 1 μm) and synaptic ribbon, but no invaginations, were observed in this fraction; these were identified as bipolar terminals.

The crude mitochondrial fraction (P2) contained myelin fragments from the optic nerve, fragmented and partially intact photoreceptor outer segments (Fig. 1*b*), and many free mitochondria. Many conventional nerve ending particles (Fig. 1*c*) from horizontal and amacrine cells were observed, but no pinched-off photoreceptor terminals were identified in this fraction. Bipolar cell terminals containing synaptic ribbons were observed. Many other particles were present which varied in size and shape: these contained numerous small electron-opaque granules (ribosomes) but no mitochondria (Fig. 1*d*) or synaptic thickenings and may have been derived from processes of the neuroglial Müller cells[18].

This preliminary morphological study of the subcellular particles produced by homogenisation of the rabbit retina shows that conventional retinal nerve endings form synaptosomes similar to those previously described for many other areas of the CNS. The photoreceptor terminals, however, are unique in their distinctive morphology and like cerebellar mossy fibre nerve endings[12], but unlike other nerve ending particles, sediment in the P1 pellet. Thus, it should be possible to obtain, for the first time, a preparation containing only isolated photoreceptor terminals. Such a preparation should be of great help in identifying the transmitter substance of the photoreceptors.

It has been proposed that an excitatory transmitter is continuously released from photoreceptors in the dark,

Table 1 Distribution of endogenous amino acids in primary fractions

	% of total recovered in P1 + P2 + SNT.			Relative specific activity (RSA)		
	P1	P2	SNT	P1	P2	SNT
GABA	20.2	10.9	68.8	0.50	0.60	1.68
Aspartate	41.5	13.9	44.5	1.08	0.76	1.03
Glutamate	21.0	6.5	70.9	0.53	0.36	1.71
Glycine	23.8	11.2	64.9	0.58	0.62	1.57

Approximately 70 mg wet weight of rabbit retinae were homogenised in 20 volumes 0.32 M sucrose (6% w/v). Each retinal homogenate was spun at 1,000*g* for 5 min to produce a crude nuclear pellet (P1). The supernatant was then centrifuged at 17,000*g* for 30 min to produce a crude mitochondrial pellet (P2) and SNT (supernatant of latter centrifugation[12]). The amino acids in these fractions were extracted with ice-cold perchloric acid (6%) and allowed to stand overnight. The extracts were taken to *p*H 4.0 with KOH and centrifuged at 0° C to remove the perchlorate precipitate. The supernatant was acidified to *p*H 2.2 with citrate buffer and the amino acids were determined by ion-exchange chromatography using an automatic amino acid analyser (Biocal BC100). Results are expressed as both % of total recovered in P1 + P2 + SNT and relative specific activity (RSA = % total recovered in fraction/% total protein recovered in fraction). Results are means of 4–6 experiments. The s.e. of the means were between 0 and 10%.

but release slows in the light, resulting in hyperpolarisation of the horizontal cell[19,20] Recently, Dowling and Ripps[21] have suggested that the photoreceptor transmitter may be aspartate or glutamate on the basis that increased Mg levels, which would be expected to reduce transmitter release, cause hyperpolarisation of second order retinal neurones Since our experiments suggest that the P1 fraction contains most of the photoreceptor terminals, we have examined the distribution of endogenous aspartate, glutamate, GABA and glycine in the primary fractions produced from the rabbit retina (Table 1) These experiments indicate that the P1 fraction contains more than 40% of the aspartate, whilst only about 20% of the other amino acids are concentrated in this fraction These results are consistent with the suggestion that aspartate may be the transmitter released from the photoreceptor terminals

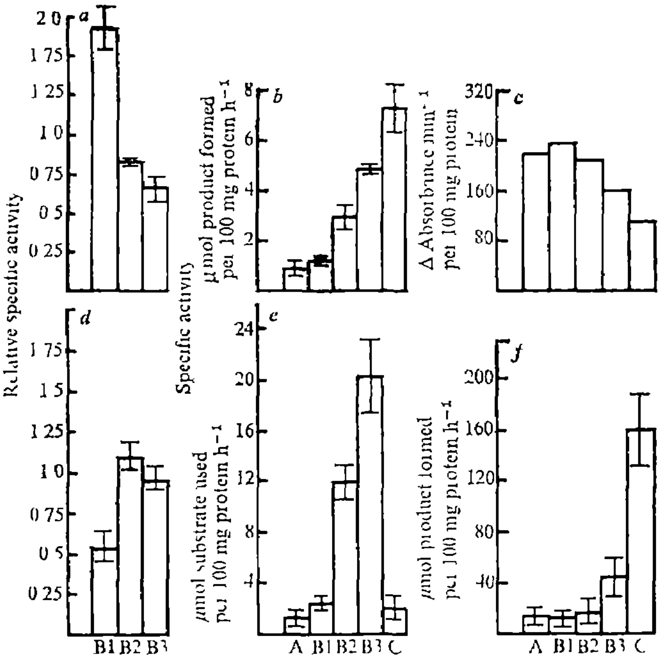

Fig 2 Distribution of enzymes, ^{3}H-dopamine, and ^{14}C-GABA sucrose gradients Retinae were incubated with a mixture of ^{3}H-dopamine (0 1 µM) and ^{14}C-GABA (0 1 µM) for 20 min at 37° C in medium containing pargyline (15 µM), EDTA (25 µM), and ascorbic acid (1 mM) These retinae, and also unincubated retinae for enzyme assays, were homogenised in 0 32 M sucrose and centrifuged at 1,000*g* for 5 min The supernatant was then spun at 17,000*g* for 30 min at 4° C to produce a crude mitochondrial pellet (P2) This pellet was resuspended in 0 32 M sucrose and layered on to the top of a four step discontinuous sucrose gradient (equal volumes of 1 4, 1 2, 1 0, 0 8 M sucrose) which was then centrifuged at 100,000*g* for 90 min at 4° C The five resulting fractions were collected by aspiration Fraction A being that floating on 0 8 M sucrose C sedimenting below 1 2 M sucrose The three intermediate fractions were designated B1, B2 and B3 The results are the means of 4 to 11 experiments, the vertical bars are the s e of the mean *a*, ^{3}H-Dopamine, *b*, MAO, *c*, LDH, *d* ^{14}C-GABA, *e*, GAD, *f*, GABA-T

We have not yet been able to examine the morphology of the particles present in the various fractions obtained by density gradient centrifugation of the crude mitochondrial pellet (P2) The mitochondrial enzymes MAO and GABA-T however were predominantly localised in the pellet (C) (Fig 2) The GAD activity was found predominantly in fraction B2 and B3 Since previous studies have shown that these fractions are rich in synaptosomes, and that GAD is concentrated in nerve endings[22-24], it seems likely that the retinal particles sedimenting in B2 and B3 represent 'gabainergic nerve endings' Particles in these same fractions also accumulated most of the ^{14}C-GABA on the gradients, this coupled with previous electron microscope autoradiographical studies on cortical homogenates which have shown that labelled GABA is accumulated by nerve terminals[25], suggests that GABA-operated retinal nerve endings may also accumulate labelled GABA But we cannot at present exclude the possibility that GABA-containing glial particles also sediment in these fractions

^{3}H-Dopamine seemed to be localised preferentially in the B1 fraction suggesting that it was accumulated by slightly 'lighter' particles than those which accumulated ^{14}C-GABA These particles are also likely to be synaptosomes since autoradiographic studies of ^{3}H-dopamine uptake in the retina have only revealed neuronal uptake[3,4]

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Newly synthesised lipids incorporated into influenza virus membranes

USING sequential labelling techniques, I have found that newly synthesised as well as preformed lipids are incorporated into the membrane of the maturing influenza virus, thus contradicting suggestions that only pre-existing phospholipids[1–3] are utilised for the synthesis of enveloped viruses. This is not surprising in the light of reports that a selection mechanism exists for fatty acyl chains[4,5] (in ortho- and para-myxoviruses) and that many phospholipids[6] and neutral lipids[7] turn over rapidly in cultured animal cells.

Influenza virus can be considered a template virus—newly synthesised polypeptides are inserted into the membrane by lateral displacement of existing host cell polypeptides[8]. Studies of the origin of phospholipids have involved the use of ^{32}P-orthophosphate to measure the decay of phospholipids in the virion and infected cells. Although this technique appears to be valid for equilibrium labelling and thus for measuring the lipid composition of the virion[9], attempts to chase ^{32}P-orthophosphate from phospholipids in monolayer cultures have been unsuccessful. Since ^{32}P can exchange rapidly or compartmentalise into organic pools which turn over slowly, data for the turnover of phospholipids in chick embryo fibroblasts[7] obtained with this isotope would seem to be unreliable[8]. Using short pulses of 2-^{3}H-glycerol, Weinstein and I have already shown that the depression of synthesis of host cell lipids in virus-infected chick embryo fibroblasts depended on the multiplicity of infection, suggesting that newly synthesised lipids were incorporated into the virion[11].

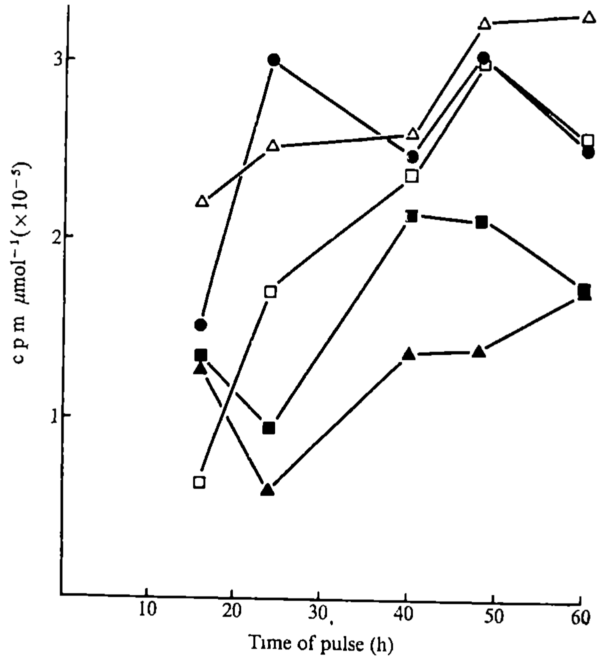

Fig 1 Equilibrium labelling of chick embryo fibroblasts plated in the presence of 0.45 μCi ml^{-1} of 2-^{14}C-glycerol. △, Phosphatidylcholine; ■, phosphatidylethanolamine; ●, phosphatidylinositol; □, phosphatidylserine; ▲, sphingomyelin.

To investigate this question further, secondary chick embryo fibroblasts were seeded on to Falcon flasks (75 cm^2) in Puck's medium containing 10% calf serum and 0.45 μCi of 2-^{14}C-glycerol (ICN, specific activity 16 mCi mmol^{-1}). Individual flasks containing about 10^7 cells were sampled at various times, lipids were extracted and separated by thin-layer chromatography and their specific activity was determined[6]. With the exception of sphingomyelin, all phospholipids were labelled to equilibrium within 48 h (Fig 1). To achieve maximal incorporation of label, confluent cells (at 48 h) were washed with KIR[6] and cells starved for 16 h in minimal Eagle's medium with 0.1% fatty-acid-poor bovine serum albumin (MEM-FAPBSA) containing 0.45 μCi ml^{-1} of 2-^{14}C-glycerol per ml. This step further increased the incorporation of ^{14}C-glycerol by 50% (12–16 h were needed to achieve further equilibrium labelling, in the absence of serum). Monolayers were then washed with ice-cold KIR and infected with 15–20 plaque-forming units (PFU) per cell of influenza virus (Ao/WSN/34 strain) by adsorption at 4° C for 90 min. The virus inoculum was removed and the cells were pulsed with 1 μCi ml^{-1} of 2-^{3}H-glycerol (New England Nuclear Corp., specific activity 8.8 Ci mmol^{-1}) in MEM-FAPBSA for 16 h. Virus was collected after 16 h, the purification of virus and extraction and separation of lipids is described in the legend of Table 1.

Table 1 Incorporation of 2-^{3}H-glycerol into influenza virions derived from prelabelled cells

	Phospholipids c p m μmol^{-1} Pi ^{14}C	^{3}H	^{3}H/^{14}C*
Lysophosphatidylcholine	14.8	10.4	0.7 (±0.1)
Phosphatidylcholine	315	312	1.0 (±0.1)
Phosphatidylserine	132	39	0.3 (NC)
Phosphatidylethanolamine	127	53	0.4 (±0.1)
Phosphatidylinositol	19	12	0.6 (±0.1)
Sphingomyelin	132	34	0.3 (NC)
	Neutral lipids c p m μmol^{-1} ^{14}C	^{3}H	^{3}H/^{14}C*
Monoglycerides	208	83	0.4 (±0.1)
Free fatty acids	137	70	0.5 (±0.1)
Cholesterol	1964	200	0.1 (NC)
Diglycerides	172	241	1.4 (±0.15)
Triglycerides	91	227	2.5 (±0.2)
Cholesterol esters	250	83	0.3 (NC)

Doubly labelled virus was mixed with cold purified egg-propagated influenza virus, containing the equivalent of 300 μg of phospholipid, and purified by adsorption and elution from BaSO$_4$, two cycles of differential centrifugation and by banding (twice) in preformed gradients of potassium tartrate[12]. In a separate experiment, isotope dilution techniques[12] with ^{32}P revealed <0.1% contamination by host cell phospholipids as judged by Čerenkov radiation[13]. The extraction and quantification of labelled virus was as previously described[6,11]. Specific activities were determined after subtraction of the mean values obtained from duplicate runs of individual lipids obtained from cold purified virus from that of labelled virus plus an equivalent amount of cold virus which was separated by thin-layer chromatography. Corrections for background and overlap of channels was done directly in an Intertechnique spectrometer.

NC, no change. Pi, inorganic phosphate. Results represent means of three separate experiments.

*Bracketed figures represent variability of ratios between experiments.

Analysis of neutral glycerides showed that about 55–70% of di- and triglycerides were newly synthesised, whereas phosphatidylcholine contained half preformed and half newly synthesised lipid and in the case of phosphatidylethanolamine, about 30% was newly synthesised. Although glycerol labels cholesterol randomly, most of it appears to be synthesised before infection, confirming that this lipid turns over slowly.

These experiments provide direct evidence that newly synthesised lipids are incorporated into the virus (after infection). Since lipids are synthesised at the endoplasmic reticulum and transported to the cell surface membrane, it would appear that they must be transported to sites of viral membrane biogenesis which are 'hot spots'[14] for the integration of newly synthesised lipids as well as polypeptides coded for by the virus. The mechanism involved is unclear, but the most likely candidates for selective binding and transport of lipids to the sites of viral biogenesis are the envelope polypeptides and/or the 'M' protein[8]. These informational molecules determine the number and species of fatty acyl chains incorporated and provide a system for integration of lipids into membranes[8].

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Volatilisation of mercury and organomercurials determined by inducible R-factor systems in enteric bacteria

WE have screened a large number of R plasmid-bearing *Escherichia coli* and obtained a few that confer resistance to the organomercurials phenylmercuric acetate (PMA) and methylmercuric chloride (MMA) Resistance to cationic Hg(II) in *E coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* has invariably been associated with plasmids that also mediate resistance to various antibiotics (R plasmids)[1–6] The mechanism of mercury resistance is the enzymatic reduction of Hg(II) to Hg(0), which is volatile[2,7,8] Previously, no organomercurial-reducing *E coli* strains have been reported The only organomercurial-reducing strain studied in detail is a PMA-resistant soil pseudomonad[7–9] As organomercurials including PMA are a human health problem[10], it will be of interest to see if the mercury(ial) resistance of the bacteria in our alimentary canal influences the fate of ingested mercury(ials)

Starting with the Hammersmith Hospital collection[11,12] wherein over 800 enteric plasmids have been isolated and transferred into a common 'host' organism, an *E coli* K-12 strain J53, each of the plasmid-bearing strains was tested by disk assay for resistance to both mercuric chloride (100 nmol per disk) and to phenylmercuric acetate (7 nmol per disk) A series of over 30 mercuric-resistant strains including some also resistant to PMA were tested for the ability to volatilise radioactive ^{203}Hg(II) and ^{203}Hg-PMA

The distinction between sensitive and resistant strains is absolute with regard to volatilisation of Hg(II) and PMA (Fig 1) Sensitive bacteria without plasmids such as strains J53-1 and AB1932-1 show no detectable loss of ^{203}Hg(II) or ^{203}Hg-PMA from the medium during 90 min incubation at 36 C Strains harbouring R-factors that confer Hg(II)-resistance but not PMA-resistance volatilise only ^{203}Hg(II) and not ^{203}Hg-PMA (for example, strain J53(R222)) and strains that are resistant to both Hg(II) and PMA (such as strain J53(471a)) cause the volatilisation of both compounds

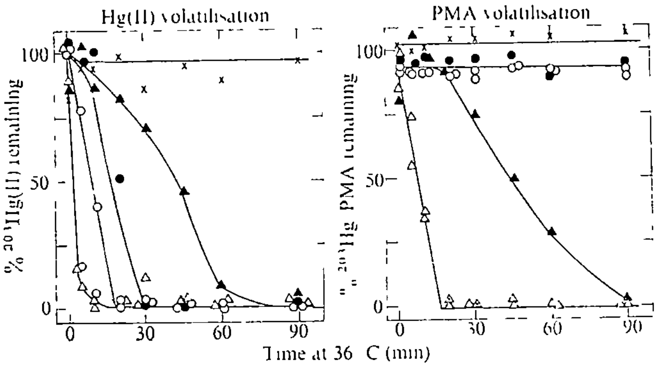

Fig. 1 Volatilisation of ^{203}Hg(II) and ^{203}Hg-PMA Cells were grown overnight at 37° C in tryptone broth (8 g Difco Bacto-tryptone, 5 g NaCl l^{-1} deionised water) or in tryptone supplemented with 10 μM $HgCl_2$ or 1 μM PMA In the morning, the cultures were diluted tenfold into the same medium and when growth reached 75 Klett units turbidity (number 54 filter, Klett-Summerson colorimeter), a third addition of Hg(II) or PMA was made to the induced cultures After growth to 125 Klett units (230 μg dry weight ml^{-1}), the cells were collected by centrifugation at 6,000*g* for 10 min at 20° C and resuspended at 2 3 mg ml^{-1} (dry weight) in 0 01 M $NaPO_4$ buffer, *p*H 7 3 Cells were diluted elevenfold into an assay buffer containing final concentrations of 0 01 M $NaPO_4$ buffer, *p*H 7 3, 5×10^{-4} M Na_2EDTA, 10^{-3} M β-mercaptoethanol, 2×10^{-4}M $MgCl_2$ and either, *a*, 10 μM $^{203}Hg(NO_3)_2$ or *b*, 1 0 μM ^{203}Hg-PMA 20 μl samples were removed periodically and counted by liquid scintillation spectroscopy[2] The reaction mixtures (220 μl) were shaken at 200 r p m in 10 × 75 mm test tubes in a 36° C water bath ×, Uninduced J53-1, ●, uninduced J53(R222), ○, Hg(II)-induced J53(R222), ⊙, PMA-induced J53(R222), ▲ uninduced J53(471a), △ Hg(II)- induced J53(471a), △ PMA-induced J53(471a)

The results of a series of such experiments are summarised in Table 1 along with the antibiotic resistance pattern of each plasmid, the compatibility groups, and the bacterial species from which the plasmids were isolated A small number of R factors from other sources and some hospital isolates were also tested All of the 47 strains with mercury volatilising activity listed in Table 1 are inducible for this activity This is seen in Fig 1, where the initial rate of loss of ^{203}Hg(II) is five to ten times more rapid with bacteria that have been previously exposed to 10 μM $Hg(NO_3)_2$ or 1 μM PMA With the strains harbouring the first eight plasmids that confer activity toward both Hg(II) and PMA, growth on either substrate induced the immediate rapid volatilisation of both—that is, a single system appears to be functioning or there is at least cross induction With 38 of the remaining 39 Hg(II)-resistant strains, growth in the presence of 1 μM PMA induces the rapid volatilisation of ^{203}Hg(II), even though ^{203}Hg-PMA is not volatilised (that is, PMA appears to be a gratuitous inducer but not a substrate for the system)

As well as the two most common patterns (exemplified in Fig 1 by plasmids R471a and R222), we have found a number of exceptional induction-volatilisation patterns, each of which is reproducible in repeated experiments with separate single colony isolates First, strains with R factors R826 and R828 did not induce volatilisation of PMA in the assay conditions, although when pregrown with either Hg(II) or PMA, these strains rapidly volatilised PMA Second, strains J53(R40a), RN180 and RN181 showed low level volatilisation of ^{203}Hg-PMA (±,< 50% of 1 μM PMA volatilised in 90 min at 36° C) as opposed to > 90% loss in 30 min with J54(R471a) (Fig 1) Previous growth on 1 μM PMA induced rapid volatilisation of 10 μM Hg(II) by strains J53(R40a), RN180 and RN181, but little or no activity toward PMA These strains belong to the class of organomercurial-reducing strains, but they are quantitatively very different from the first eight plasmid-containing strains in Table 1 Third, with strain J53(R192) exposure to 1 μM PMA strongly inhibited (to less than one-fifth of the uninduced level) the ability of the cells to volatilise ^{203}Hg(II) Higher levels of PMA (approaching 10 μM) and $Hg(NO_3)_2$ (approaching 20 μM) have this effect on all the strains Fourth, the strains carrying R factors R391 and R705 do not show induction of ^{203}Hg(II) volatilising activity during 90 min at 36° C in the buffer assay conditions

We used phenylmercuric acetate for screening R factor-containing strains for resistance to organomercurials Recently, we have measured the volatilisation of ^{203}Hg and ^{14}C from radioactive methylmercuric chloride when exposed to induced cells of J53(R471a), J53(R286) and J53-1(R831), but not when induced cells of these strains were used, nor with J53 derivatives with plasmids R15, R192, or R391 or the plasmidless J53-1 (all pre-exposed to Hg(II)) Strain RN180 induced with Hg(II) showed low level but significant volatilisation of ^{203}Hg-methylmercuric chloride

Thirty-one of the mercury-resistant strains in Table 1 contain plasmids transferred into the common genetic background of K-12 derivative J53 Plasmids JJ1, U150 and U305 were transferred into another common K-12 derivative AB1932-1 (ref 13) and have been previously shown to volatilise Hg(II)[2,4] In terms of mercury resistance and volatilisation, AB1932-1(R15) is indistinguishable from J53-1(R15) The four RN plasmid-bearing mercury-resistant strains originated from Chabbert[14] in Paris Two of

Table 1 Mercury and mercurial resistance R factors

Strain	Uninduced		Hg (II) grown		PMA grown		Plasmid characteristics		Origin
			Volatilisation of						
	Hg (II)	PMA	Hg(II)	PMA	Hg(II)	PMA	Antibiotic resistances	Compatibility group	
J53–1	−	−	sensitive		sensitive		None		
J53 (R471a)	+	+	+	+	+	+	A, Cb, Cp, Tc	L	*Serratia marcescens* (Boston, Mass)
J53 (R473)	+	+	+	+	+	+	A, Cb, Cp	L	*Serratia marcescens* (Boston, Mass)
J53 (R826)	+	−	+	+	+	+	A, Cb, Cm, Gm Km, Sm, Tc	S	*Serratia marcescens* (Toulon, France)
J53 (R828)	+	−	+	+	+	+	A, Cb Cm, Gm Km, Sm, Tc	S	*Serratia marcescens* (Toulon, France)
J53-1 (R830a)	+	+	+	+	+	+	Km, Sm, Tc	L	*Serratia marcescens* (Boston, Mass)
J53-1 (R831)	+	+	+	+	+	+	Km, Sm, Tc	L	*Serratia marcescens* (Boston, Mass)
J53 (R832)	+	+	+	+	+	+	A, Cb, Km, Tc	L	*Serratia marcescens* (Boston, Mass)
J53 (JR211)	+	+	+	+	+	+	A, Cb, Cm, Gm Km, Sm, Su	A–C	*Klebsiella aerogenes*
J53-1 (R15)	+	−	+	−	+	−	Sm, Su, Sp, Su	N	Watanabe (Japan) (ref 11)
J53 (R28)	+	−	+	−	−	+	Sm, Sp, Tc	FII	(ref 11)
J53 (R40a)	+	−	+	±	+	−	A, Cb, Cp, Km, Su	A–C	*Pseudomonas aeruginosa*
J53 (R52)	+	−	+	−	+	−	Sm, Sp, Tc	FII	(ref 11)
J53 (R57b)	+	−	+	−	+	−	A, Cb, Cm, Gm, Km, Su	A–C	*Klebsiella pneumoniae*
J53 (R192)	+	−	+	−	−	−	Cm, Tc	FII	(ref 11)
J53 (R222)	+	−	+	−	+	−	Cm, Sm, Su, Tc	FII	Watanabe (Japan) (ref 11)
J53-1 (R312)	+	−	+	−	+	−	Cm, Sm, Sp	FII	(ref 11)
J53-1 (R348F)	+	−	+	−	+	−	Cm, Km, Sp, Tc	FII	(ref 11)
J53 (R391)	−	−	+	−	+	−	Km, Tc	J	*Proteus rettgeri* (South Africa)
J53-1 (R401)	+	−	+	−	+	−	A, Cb, Sm	T	*Proteus rettgeri* (South Africa)
J53-1 (R455)	+	−	+	−	+	−	A, Cb, Cm, Sm, Su, Tc	FI	*Proteus morganii* (South Africa) (ref 11)
J53 (R700)	+	−	+	−	+	−	Su	A–C	*Providencia* (Georgia, U S A)
J53-1 (R702)	+	−	+	−	+	−	Km, Sm, Su, Tc	P	*Proteus mirabilis* (New Jersey)
J53-1 (R705)	−	−	+	−	+	−	Km, Tc	J	*Proteus vulgaris* (South Africa)
J53 (R718)	+	−	+	−	+	−	A, Cb, Cp, Km, Su, Tc	A–C	*Providencia* (Canada)
J53-1 (R725)	+	−	+	−	+	−	Cm, Sm, Su, Tc	O	*Shigella dysenteriae* (Mexico)
J53 (R754)	+	−	+	−	+	−	A, Cb, Cm, Cp, Sm, Su		*Proteus mirabilis* (Thailand)
J53 (R938)	+	−	+	−	+	−	A, Cb, Cm, Cp, Km, Sm, Tc, Su	A–C	*Serratia marcescens*
J53-1 (JR72)	+	−	+	−	+	−	Cb, Cm, Km, Sm, Sp, Tc	FII	Julian Davies (Wisconsin)
J53-1 (RH28)	+	−	+	−	+	−	A, Cb, Cm, Cp, Tc	S	*Serratia marcescens*
J53-1 (RIP69)	+	−	+	−	+	−	A, Cb, Cp, Km, Tc	M	Chabbert (Inst Pasteur, Paris) (ref 16)
J53 (RIP135)	+	−	+	−	+	−	A, Cb, Cp, Gm, Sm, Su, Tc	M	Chabbert (Inst Pasteur, Paris) (ref 16)
AB1932-1	−	−	sensitive		sensitive		None		(refs 2 and 13)
AB1932-1 (JJ1)	+	−	+	−		−	Cm, Sm, Sp, Su	FII	*E coli* (ref 13)
AB1932-1 (R15)	+	−	+	−	+	−	Sm	N	Introduced by R Hedges
AB1932-1 (U150)	+	−	+	−	+	−	A, Sm, Su, Tc		(ref 4)
AB1932-1 (U305)	+	−	+	−	+	−	Cm, Sm, Tc		(ref 4)
CR34 (R100-1)	+	−	+	−	+	−	Cm, Sm, Tc		
RN180	+	−	+	±	+	±	A, Cb, Km, Su	A–C	*Providencia*, Chabbert No 13–84
RN181	+	−	+	±	+	±	A, Cb, Cm, Cp, Km, Sm, Su, Tc	A–C	*Providencia*, Chabbert No 13–84
RN182	+	−	+	−	+	−	Cm, Sm, Su		*Salmonella paratyphi B* (ref 14)
RN184	+	−	+	−	+	−	A, Cb, Cm, Cp, Sm, Su		*Salmonella*, ref 17
DG21	+	−	+	−	+	−	Cp, Sm, Tc		*E coli*, Rochester, New York
DG35	+	−	+	−	+	−	A, Cb, Cm, Km, Sm, Tc		David Groves (ref 15)
DG36	+	−	+	−	+	−	A, Cb, Cm, Km Sm, Tc		
B36	+	−	+	−	+	−	A, Cb, Cm, Sm, Tc		
J71	+	−	+	−	+	−	A, Cb, Cm, Km Sm, Tc		*E coli*, St Louis, Missouri
J82	+	−	+	−	+	−	A, Cb, Cp, Tc		
BU9990	+	−	+	−	+	−	Sm, Tc		

The plasmids in strains J53 or J53-1 (a derivative of J53 with a chromosomal mutation to nalidixic acid-resistance) come from the Hammersmith Hospital collection[11,12] R factors R15, R28, R52, R192, R222, R312 and R348F have been previously reported[11] as mercury resistant Plasmid RIP69 has been described[16] and was assigned to compatibility class 7, later renamed group M (ref 12) Compatibility groups 'L' and 'S' will be described shortly[18] Plasmid R15 was introduced by conjugation into an acridine-orange 'cured' plasmid-less derivative of AB1932-1 (J31), Su resistance was not tested after transfer Plasmid R100 is a synonym for plasmid R222 [17], Su resistance and compatibility group were not tested with CR34 (R100-1) The symbols for antibiotic resistances are A, ampicillin, Cb, carbenacillin, Cp, cephalothin, Cm, chloramphenicol, Gm, gentamycin, Km, kanamycin, Sm, streptomycin, Sp, spectinomycin, Su, sulphonamide, Tc, tetracycline The antibiotic resistances are those tested in this study and therefore incomplete, the resistance to the penicillin class of antibiotics does not necessitate several enzymic mechanisms these seem identical to our most common class of plasmids conferring only mercury resistance, represented in Fig. 1 by R222. The other two, RN180 and RN181, represent an exceptional class. A series of hospital isolates from Rochester, New York (DG21, DG35 and DG36)[13] and from St Louis (J71, J82, B36 and BU9990) complete our current collection of mercury volatilising *E. coli*. In these strains, the plasmid nature of the mercury resistance has yet to be shown but all are multiply antibiotic resistant. The frequency of mercury resistance amongst the new St Louis isolates was 4/126 (4 of the 25 new antibiotic resistant isolates). Approximately 200 of the 800 plasmids from various enteric organisms that have been transferred into J53 confer resistance to Hg(II). The first seven of the plasmids conferring resistance to PMA and Hg(II) originated in *Serratia marcescens* isolates. Five belonged to the 'L' incompatibility group and came from Boston, Massachusetts; the other two were group 'S' and came from Toulon, France. Recently, however, strain J53 containing plasmid JR211, which belongs to the 'A-C' compatibility group and originated in *Klebsiella aerogenes*, was found to volatilise ^{203}Hg-PMA. Clearly, the frequency of mercury resistance is sufficiently high to make this a useful epidemiological marker and further studies with mercury and mercurial resistance patterns should help in elucidating relationships between plasmids.

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Unidirectional replication of plasmid ColE1 DNA

COLICINOGENIC plasmid E1 (ColE1) has been isolated from *Escherichia coli* both as a supercoiled DNA molecule of 4.2×10^6 molecular weight and as a supercoiled DNA-protein relaxation complex[1]. Treatment with certain agents that denature protein structure induces conversion of the supercoiled ColE1 DNA-protein complex to an open circular (relaxed) DNA form with one single strand break specifically in the poly (U,G) binding or 'Crick' strand[2,3]. It has been shown that the EcoR1 restriction endonuclease cleaves ColE1 DNA at a single site. Using that site as a reference point it was demonstrated that the single strand break in the open circular product of induced relaxation is located at a unique site, approximately 19% of the length of ColE1 DNA from one end of the EcoR1 cleaved molecule[4]. Only certain restriction endonucleases have previously been shown to cleave DNA at a specific nucleotide sequence and the result of their action is a double strand break[5–7].

Replicating ColE1 DNA molecules have been isolated from *E. coli* cells and examined with respect to their physicochemical properties (L. K. Williams S. Sato, R. W. Leavitt, and D.R.H., manuscript in preparation). The parental strands of these ColE1 molecules, like those of replicating SV40 molecules[8,9], are covalently closed as determined by their broad distribution between the densities of supercoiled and open circular DNA in caesium chloride–ethidium bromide density gradients, by their rapid sedimentation in alkaline sucrose density gradients and by their appearance in the electron microscope as partially supercoiled, partially open structures. Fareed *et al.*[10] used the single EcoR1 cleavage site in SV40 DNA as a reference point to determine that SV40 DNA replication proceeds bidirectionally from a unique origin. We describe here an analysis of EcoR1 cleaved replicating ColE1 DNA molecules. Evidence is presented that ColE1 DNA replication proceeds unidirectionally from an origin whose location is indistinguishable from the site of the single-strand break present in the open circular DNA of the relaxed ColE1 complex.

A lysate of *E. coli* JC411 cells, colicinogenic for the plasmid ColE1, prelabelled with ^{14}C-thymine and pulse labelled with ^{3}H-thymidine was centrifuged to equilibrium in a caesium chloride–ethidium bromide density gradient. The pulse labelled DNA was separated into two pools, one comprising ^{3}H-DNA of a density intermediate between that of supercoiled ColE1 DNA and open circular or linear DNA and the second comprising ^{3}H-DNA of a density equal to that of open circular or linear DNA. ColE1 replicating intermediates in the less dense pool consist of molecules that have replicated to a greater extent than the replicative forms in the intermediate density pool of DNA (L. K., P. H. Williams, S. Sato, R. W. Leavitt and D.R.H., manuscript in preparation). Similar observations have been made for replicating SV40 molecules[8,9]. To purify the ColE1 DNA further, each pool was centrifuged on preparative sucrose density gradients and the ^{3}H-DNA which sedimented as rapidly or more rapidly than 23S DNA (the sedimentation coefficient of supercoiled ColE1 DNA) from each preparative gradient was pooled separately.

A sample of each of the two pools was treated with the EcoR1 endonuclease and analysed by centrifugation through 5–20% sucrose density gradients. Figure 1*a* shows that before cleavage the ^{3}H-thymidine-labelled pool of DNA originally derived from the intermediate density peak in the caesium chloride–ethidium bromide density gradient sedimented with coefficients of 23–34S. There is a peak of the prelabelled ^{14}C-thymine-labelled DNA at the 23S position, presumably arising by contamination from the supercoiled peak in the preparative dye–caesium chloride density gradient. After cleavage (Fig. 1*b*) much of the ^{14}C-DNA was found in the 15S position expected for the linear ColE1 DNA. The pulse labelled ^{3}H-DNA sedimented with sedimentation coefficients between 15S and 23S after cleavage. Figure 1*c* shows that before cleavage the pool of ^{3}H-DNA derived initially for the lowest density peak in the caesium chloride–ethidium bromide density gradient sedimented rapidly between 23S and 42S with most of the ^{3}H-DNA counts between 38S and 42S. Again much of the prelabelled ^{14}C-DNA sedimented in the position of 23S supercoiled ColE1 DNA. After cleavage with the EcoR1 endonuclease (Fig. 1*d*) most of the ^{14}C-DNA sedimented at 15S while most of the ^{3}H-DNA sedimented with coefficients between 17S and 23S. The rapid sedimentation through neutral sucrose density gradients, characteristic of replicating ColE1 DNA before and after cleavage, is consistent with the observations in the electron microscope that before cleavage with EcoR1, replicating ColE1 molecules exhibit a partially supercoiled, partially open structure and that after cleavage with EcoR1 linear molecules are observed containing an internal loop of replicated DNA and two unreplicated branches. Figure 2 represents typical EcoR1-cleaved ColE1 DNA molecules in various stages of replication.

The total lengths of the linear molecules with internal loops derived from EcoR1-cleaved DNA of the intermediate and light density pools, were determined by electron microscopy. The linear molecules with internal loops were found to be of the same length as the contour length of open circular ColE1 DNA spread on the same grid to a 95% confidence level. An analysis of the changes in relative lengths of the unreplicated branches as the extent of replication changes is shown in Fig. 3 for 124 ColE1 DNA molecules that were between 3 and 70% replicated. It is clear that as replication proceeds, one unreplicated arm of the molecule, designated U_1, remains constant in size and the other unreplicated arm, U_2, progressively decreases in size. This is as expected for a unidirectional mode of replication from a fixed origin. In contrast, if replication proceeded bidirectionally from a fixed origin, both U_1 and U_2 would decrease in size with an increasing extent of replication. Extrapolation of U_1 and U_2 to 0% replication permits a determination of the position of the origin of ColE1 DNA replication. As Fig. 3 shows, the origin is calculated to be 18.3±0.9% of unit length from one EcoR1 end and 81.7% from the other.

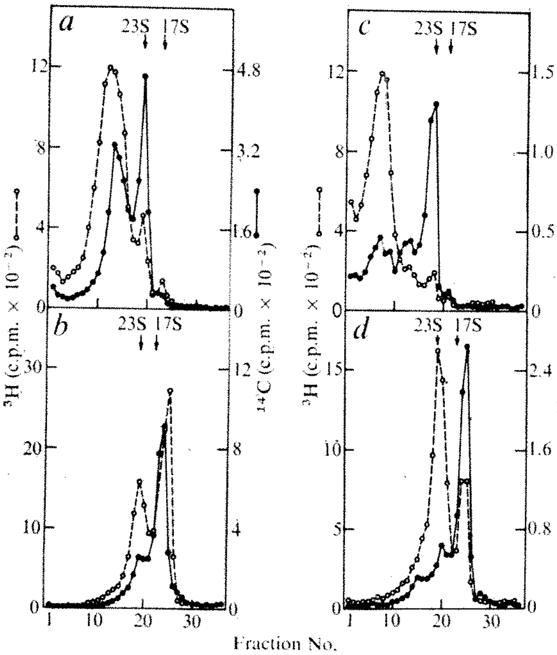

Fig. 1 Neutral sucrose sedimentation analysis of replicating ColE1 DNA molecules untreated and treated with the EcoR1 restriction endonuclease. A 500 ml culture was prelabelled uniformly with ^{14}C-thymine (0.1 μCi ml^{-1}) to a density of 5×10^8 cells ml^{-1}, collected and resuspended in medium lacking thymine and containing 3 mM cyclic AMP. After 30 min, the cells were pulsed for 30 s with ^{3}H-thymidine (10 μCi ml^{-1}) and 0.3 μg ml^{-1} of unlabelled thymidine. The cells were collected by centrifugation and lysed by the SDS-salt method[11]. The pulse label was purified by neutral sucrose gradient sedimentation and caesium chloride–ethidium bromide density gradient centrifugation (L.K., Williams, Sato, Leavitt and D.R.H., manuscript in preparation). Two peaks from the dye-buoyant density gradient containing replicating ColE1 DNA molecules, one banding at the intermediate density (*a* and *b*), the other at the light density (*c* and *d*) were sedimented through neutral 5–20% sucrose density gradients before (*a* and *c*) and after (*b* and *d*) treatment with the EcoR1 restriction endonuclease. Approximately 1 μl of an EcoR1 enzyme preparation was used for each 100 μg of ColE1 DNA in 0.1 M Tris, *p*H 7.5, 50 mM NaCl and 10 mM MgCl$_2$. After 5 min at 37° C the reaction was stopped by the addition of EDTA (final concentration of 25 mM).

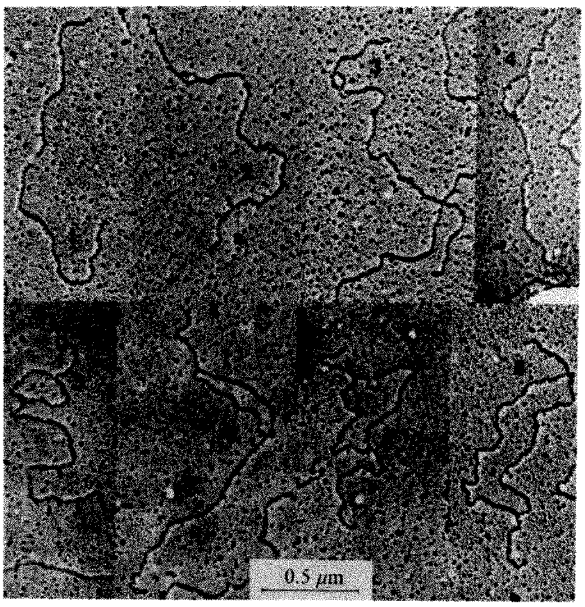

Fig. 2 Replicating ColE1 DNA molecules treated with the EcoR1 restriction endonuclease. DNA was spread by the aqueous technique of Davis *et al.*[12], rotary shadowed with platinum–palladium, and examined in the Phillips 200 electron microscope. Micrographs of selected molecules are arranged in order of increasing extent of replication. Panel 1 represents an unreplicated linear molecule.

Since replication proceeds unidirectionally, molecules greater than 81.7% replicated would be expected to have a double-Y appearance after cleavage with EcoR1. Several double Y molecules have been found in the ^{3}H-DNA derived from the light density peak in the preparative dye–caesium chloride density gradient and representative molecules are shown in Fig. 4. One of the forks of these molecules has two branches of equal size of approximately 82% of the total length of ColE1 DNA joined by a short segment to a fork with two smaller branches of equal size. As the size of the two smaller branches increases, the length of the short segment decreases. The structure of these double Y molecules is consistent with a unidirectional mode of replication from a fixed origin.

A consequence of unidirectional replication of a circular DNA molecule from a unique origin is that the origin and the termination point for replication are juxtaposed. The position of the origin-terminus for ColE1 DNA replication, 18.3±0.9% from one end of the EcoR1 cleaved molecule, is indistinguishable from the location of the single strand break present in the open circular DNA of the relaxed ColE1 DNA-protein complex (approximately 19% of unit length from one end of the EcoR1-cleaved molecule). While these data do not distinguish between the two ends of the EcoR1-treated molecule, the apparent correspondence of the nicked site and the origin-terminus implies a direct involvement of the protein components of the relaxation complex in ColE1 DNA replication.

Other plasmids studied in *E. coli*, including several different sex factors, Col, and R plasmids, have also been isolated in the form of DNA-protein relaxation complexes[13]. The relaxation event has been shown to be strand specific for ColE2 (ref. 3), F1 (ref. 14) and R6K (Y. Kupersztoch, M.A.L. and D.R.H., submitted for publication) as well as for ColE1, and it has been demonstrated that the single strand break found in the open circular DNA after induced relaxation of the ColE2 and PSC101 (ref. 4) and R6K (Y. Kupersztoch, M.A.L. and D.R.H., manuscript submitted for publication) complexes is located at a unique site in each case. The replicon hypothesis of Jacob *et al.*[15] advanced the idea that DNA replication is regulated by the action of a protein, the initiator, at the origin of replication, the replicator locus. It is possible that these specific complexes of plasmid DNA and protein reflect the basic organisation of plasmids in *E. coli* as autonomous units of replication.

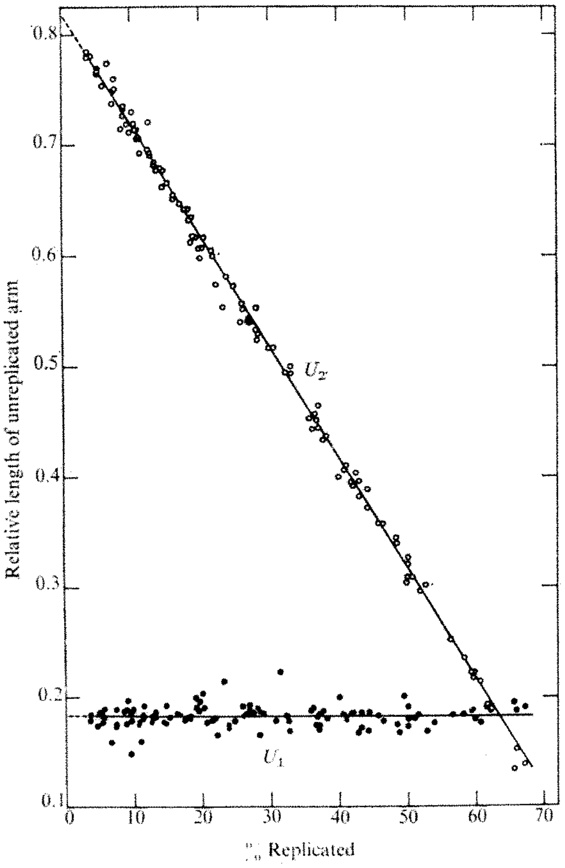

Fig. 3 Analysis of the length of the two unreplicated segments of replicating ColE1 DNA molecules after cleavage with EcoR1 as a function of the extent of replication. Replicating ColE1 DNA molecules purified and treated with the EcoR1 restriction endonuclease as described in Fig. 1 were used in this analysis. U_1 and U_2 are the unreplicated branches joining an internal loop comprised of R1 and R2. The relative lengths of U_1 and U_2 for each molecule was determined from the formula $T = U_1 + U_2 + 1/2(R_1 + R_2)$, where T = total length. The percentage replicated = $1/2\ (R_1 + R_2)/T$.

Since the strand specific break found as a result of induced relaxation is at the origin-terminus of ColE1 DNA replication, it is conceivable that the protein components of the relaxation complex could be involved in the initiation of DNA synthesis and/or in its termination. At least in the case of the ColE1 DNA-protein relaxation complex it has been shown that the protein components of the complex have novel properties that could be consistent with a direct role in DNA synthesis. The supercoiled ColE1 complex has three major protein components, whose molecular weights are approximately 60,000, 16,000 and 11,000 (M.A.L. and D.R.H., manuscript in preparation). After the induction of relaxation by treatment with sodium dodecyl sulphate (SDS), the 16,000 and 11,000 molecular weight proteins are completely released from the open circular DNA and the 60,000 molecular weight protein is found in an association, proposed to be covalent, with the broken strand (M.A.L. and D.R.H., manuscript in preparation) at its 5′ terminus (D. G. Guiney and D.R.H., manuscript in preparation). Such nicking and covalent joining *in vivo*, augmented by a capability to reseal the nicked DNA, could provide the swivel mechanism thought to be necessary to facilitate the unwinding of the parental strands of replicating DNA. Activity as a swivel mechanism might be required both before and after the initiation of DNA synthesis. A nicking and resealing mechanism could also be active at the termination point of DNA synthesis to facilitate the separation of two completed but interlocked molecules.

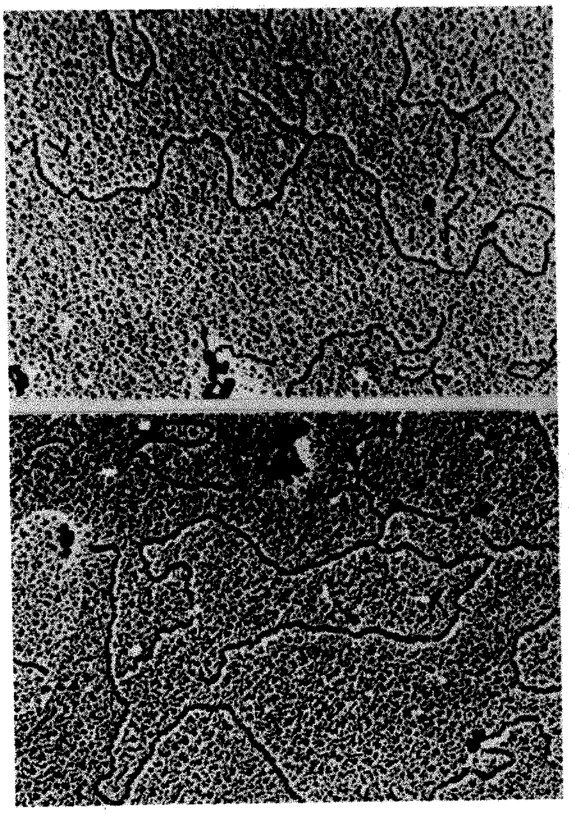

Fig. 4 Double Y structures derived from EcoR1 treatment of replicating ColE1 DNA molecules. Replicating ColE1 DNA molecules were purified and treated with EcoR1 as described in Fig. 1.

It remains to be determined whether or not unidirectional replication is an obligatory feature of the plasmid state (perhaps reflecting an essential role for relaxation complex in initiation and termination of DNA synthesis). The identification of a plasmid with the property of bidirectional DNA synthesis coupled with knowledge of the location of the site nicked by the relaxation complex could resolve the question of the action of the relaxation complex at either the origin or at the terminus of DNA replication.

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Rifampicin-resistant replication of coliphage N4 DNA

IT has recently been reported that rifampicin-sensitive RNA synthesis participates in the replication of bacterial[1,2], episome or plasmid[3–8] and phage[7–11] DNA, presumably in a primer role during initiation. A more complex model of DNA replication may, however, be provided by the development of coliphage N4 (ref. 12). Transcription of N4 chromosome is unusual in that the virus uses two new rifampicin-resistant polymerising activities[13]. Expression of the early and delayed-early genes is therefore unaffected in rifampicin-pretreated cells although infection is abortive since transcription of the late N4 genes requires the functioning of the rifampicin-sensitive *Escherichia coli* RNA-polymerase (ref. 14). Here we show that initiation and continuation of N4 DNA replication take place in conditions in which the host RNA polymerase is totally inactive and give an account of the nature of the products that are synthesised in rifampicin-pretreated cells.

As shown in Fig. 1, rifampicin (200 μg ml^{-1}) added to Hfr 3300 cells 10 min before infection, did not prevent host DNA shut-off and subsequent phage DNA replication. N4 DNA synthesis was always initiated with a 3–5 min delay and the rate of ^{3}H-thymidine incorporation reached from 50–60% of that observed during normal infection. The bulk of the newly produced DNA is viral, since all of the DNA-negative *amber* mutants[15] tested failed to induce DNA synthesis. The effect on phage-coded macromolecular syntheses of adding rifampicin at different times before infection suggests that the reduction in the rate of DNA synthesis is caused by the decreased protein synthesis rather than by a direct effect of the drug on DNA replication (Fig. 2). As practically no incorporation of ^{3}H-uridine into RNA was observed in uninfected cells pretreated for 10 min with rifampicin it can be concluded that N4-directed DNA production can occur in the total absence of transcription by the host RNA-polymerase. It should be stressed, however, that N4-infected rifampicin-pretreated cells do synthesise large amounts of RNA (Fig. 2). Rothman-Denes and Schito have demonstrated that this rifampicin-resistant RNA hybridises specifically only to N4 DNA (ref. 13).

The nature of the DNA synthesised in the presence of rifampicin was subsequently investigated. Earlier studies showed that N4 parental DNA rapidly attaches to the cell membrane and that most of the ^{3}H-thymidine incorporated in a 1 min pulse administered at any time after the onset of phage DNA synthesis is complexed to fast sedimenting bacterial sites[16] where replication takes place (intermediate I)[17]. Maturation of the replicated DNA to the 40S molecule contained in the virion[18] requires the expression of late phage functions. Infection performed in rifampicin-pretreated cells resulted in the association of approximately 85% of the ^{32}P-labelled parental N4 chromosome with the cell membrane (not shown) implying that RNA synthesis mediated by the host RNA polymerase is not required for attachment. The sedimentation profile of the pulse-labelled DNA synthesised in the presence of the drug is furthermore undistinguishable from that observed under normal conditions, indicating that rifampicin does not prevent the parental genome from replicating in association with the correct bacterial site (Fig. 3*a* and *b*). When the fate of the

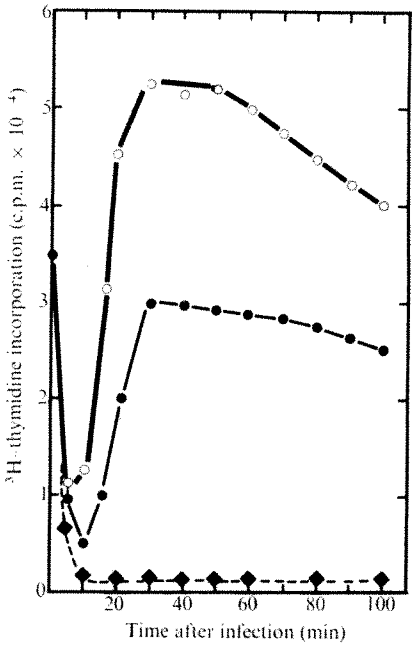

Fig. 1 Effect of rifampicin on N4 DNA synthesis. *E. coli* K12 strain Hfr 3300 was grown at 37° C in M9S casamino-acids-glucose medium[12] to a density of about 2×10^8 cells ml^{-1}. The culture was divided into several portions of equal volume (10 ml). One portion served as a control while 200 μg ml^{-1} of rifampicin (Lepetit) was added to the other portions. After shaking at 37° C for 10 min, wild type or *amber* 8, 12, 15, 23 or 25 DNA-negative (DO) N4 mutants were added separately at a multiplicity of infection of 10. At specified times thereafter 0.2 ml aliquots were removed and incubated for 2 min with methyl-^{3}H-thymidine (10 μCi ml^{-1}, 14 Ci mmol^{-1}, Amersham, England). At the end of the pulse 0.05 ml samples were precipitated with trichloroacetic acid and counted in toluene PPO as previously detailed[15]. ○, N4 wild-type-infected culture, untreated; ●, N4 wild-type infected culture, pretreated with rifampicin; ◆ DO-*amber* mutant-infected cultures, pretreated with rifampicin.

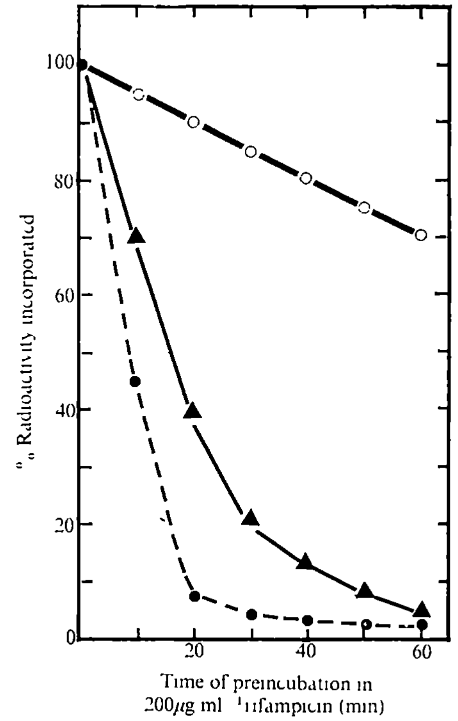

Fig 2 Rate of macromolecular syntheses in Hfr 3300 cells incubated with rifampicin for different lengths of time and infected with coliphage N4 Cells were grown according to the procedure described in the legend of Fig 1 Rifampicin (200 μg ml^{-1}) was added to different cultures 0, 10, 20, 30, 40, 50 and 60 min before infection with N4 at a multiplicity of 10 To measure incorporation of labelled precursor into DNA, 0 2 ml samples were removed 20 min after the addition of the phage and pulsed for 2 min with methyl-^{3}H-thymidine (10 μCi ml^{-1}) RNA synthesis was assessed by pulsing 0 2 ml aliquots from 6–8 min postinfection with ^{3}H-uridine (5 μCi ml^{-1}, 41 Ci mmol^{-1}, Amersham) and protein synthesis was determined by labelling for 3 min 0 2 ml portions, taken 5 min after infection, with ^{3}H-leucine (20 μCi ml^{-1}, 0 125 Ci mmol^{-1}, Amersham) The TCA-insoluble radioactivity incorporated was measured as described[15] Values are plotted as a percentage of the radioactivity incorporated by Hfr 3300 cells treated with rifampicin at time zero of infection On the ordinate 100 corresponds to 41,400 c p m for ^{3}H-thymidine, 67,000 c p m for ^{3}H-uridine and 8,300 c p m for ^{3}H-leucine ▲, Isotope incorporation into DNA, ○, into RNA, ●, into protein

DNA contained in intermediate I was analysed by chasing the radioactivity incorporated during the pulse with an excess of unlabelled thymidine, strikingly different results were observed depending on whether intermediate I was formed in the presence of rifampicin or not As shown in Fig 3*c* in normal conditions most of the pulse-labelled DNA in intermediate I was released as material sedimenting with a rate characteristic of mature N4 DNA after a chase of 20 min, whereas none of the DNA species synthesised in drug-pretreated cells could be chased into slowly sedimenting material (Fig 3*d*) Analysis by neutral or alkaline sucrose density gradient centrifugation of the DNA accumulated during continuous labelling of infected cells confirmed that upon expression of late phage functions the newly replicated DNA gives rise to molecules possessing the same hydrodynamic properties shown by N4 mature polynucleotide chains (Fig 4*a* and *c*) Detachment and maturation of N4 DNA are possibly interdependent events since in rifampicin-pretreated cells not only did detachment from the bacterial site fail to occur (Fig 4*b*) but the polynucleotide chains accumulated in intermediate I, upon denaturation by alkali migrated as an heterogeneous peak with much of the material sedimenting faster than single strands of mature DNA (Fig 4*d*) The lack of transcription, in the presence of the antibiotic, of the late phage genes[13] which code for maturing factors could explain the accumulation of the fast sedimenting DNA species

The extended synthesis of viral DNA in cells pretreated with rifampicin (Fig 1) indicates that the host RNA-polymerase is not required in the initiation and elongation reactions of N4 chromosome replication These findings are in contrast with those obtained with other host–phage systems in which rifampicin-sensitive transcription has been shown to be a prerequisite for at least the initiation of replication[7–11] One might therefore assume either that RNA synthesis in general is dispensable for N4 DNA replication or that RNA priming is required for initiation but is carried out by a system distinguishable from the host RNA-

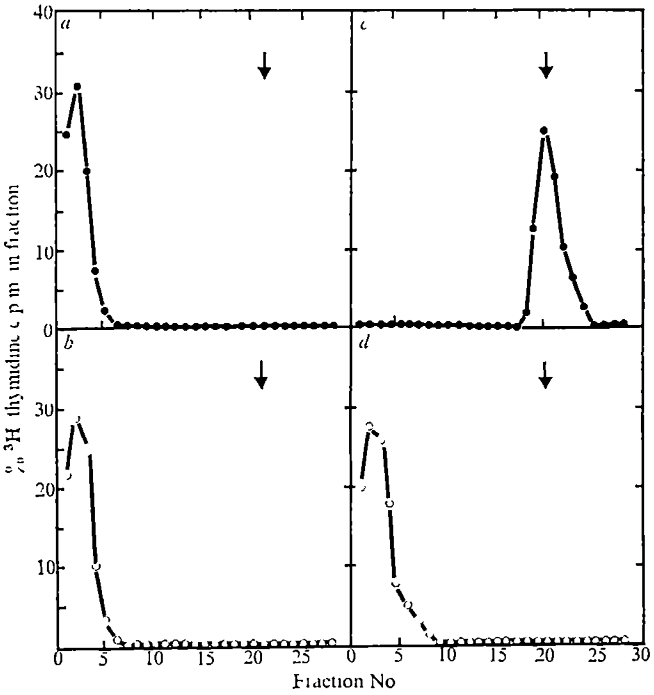

Fig 3 Sedimentation analysis of N4 DNA synthesised in control cells and in cells pretreated with rifampicin *E coli* Hfr 3300 was grown at 37° C in M9S with shaking When the cell density reached 2 5 × 10^8 cells ml^{-1} the culture was divided into two portions One portion was left untreated and served as a control and 200 μg ml^{-1} of rifampicin was added to the other aliquot After 10 min incubation at 37° C N4 was added at a multiplicity of 10 Newly synthesised phage DNA was labelled from 20–22 min after infection by the addition of methyl-^{3}H-thymidine (14 Ci mmol^{-1}) to a final concentration of 20 μCi ml^{-1} At the end of the labelling period a 2 ml sample was taken and processed as described below To the remaining culture unlabelled thymidine was added to a final concentration of 2 0 mg ml^{-1} At 40 min after infection a sample of cells was centrifuged and gently lysed by the Tris-lysozyme-EDTA-detergent method described by Hallick Boyce and Echols[21] Portions of the cell lysates (0 15 ml) were mixed with ^{32}P-N4 DNA (ref 17) and centrifuged in a 4 2 ml of 5–20% sucrose gradient overlaying a 0 3 ml 1 7 g ml^{-1} caesium chloride shelf Sucrose solutions were made up in 0 02 M Tris-HCl, *p*H 8 0, containing 0 01 M EDTA Centrifugation was in the Spinco SW50 rotor for 60 min at 35,000 r p m and 5° C Fractions (about 0 15 ml) were collected from the bottom of the tube and the acid-insoluble radioactivity in a 0 05 ml sample was determined Overall recoveries of ^{3}H were greater than 85% Labelled N4 DNA found at the CsCl-sucrose underlayer is defined as Intermediate I The arrows indicate the position to which marker ^{32}P-N4 DNA sediments *a*, Sedimentation pattern of pulse-labelled N4 DNA, control cells, *b*, sedimentation pattern of pulse-labelled N4 DNA cells pretreated with rifampicin, *c*, fate of pulse-labelled N4 DNA after a 20 min chase with unlabelled thymidine untreated cells *d*, fate of pulse-labelled N4 DNA cells pretreated with rifampicin

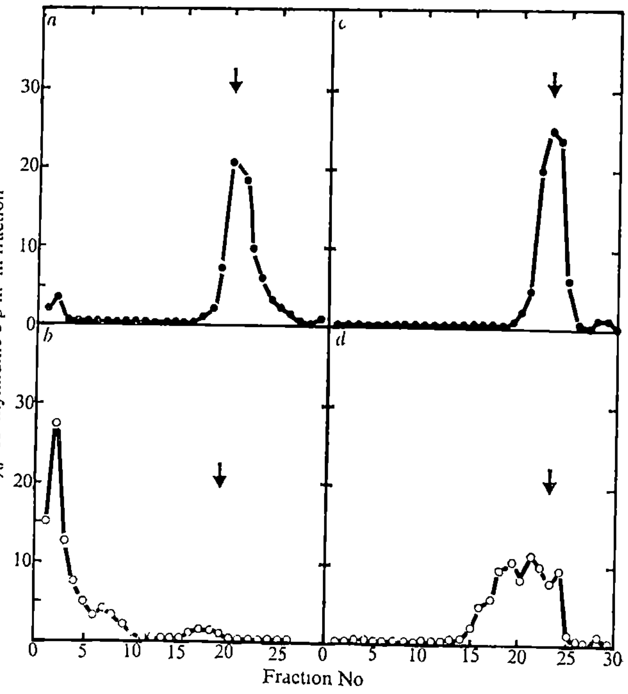

Fig 4 Sedimentation analysis of N4 DNA labelled continuously in control cells and in cells pretreated with rifampicin Hfr 3300 was grown as described in the legend of Fig 1 At a cell density of 2×10⁸ cells ml⁻¹ the culture was divided into two aliquots One portion served as a control while 200 μg ml ⁻¹ of rifampicin was added to the other portion After 10 min incubation at 37° C N4 was added at a multiplicity of 10 Phage DNA was continuously labelled from 20 to 40 min after infection by the addition of methyl-^{3}H-thymidine (14 Ci mmol^{-1}) to a final concentration of 5 μCi ml $^{-1}$ At the end of the labelling period, samples were taken and DNA was gently extracted as specified in the legend of Fig 3 Portions of the cell lysate (0 15 ml) were mixed with ^{32}P–N4 DNA and centrifuged in neutral or alkaline sucrose gradients Alkaline sucrose solutions were made up in 0 001 M EDTA and 0 3 M NaOH Conditions of sedimentation and analysis were the same as those described in Fig 3 *a* and *c*, Patterns of sedimentation of N4 DNA labelled continuously from 20–40 min after infection in control cells *a*, Neutral sucrose gradient, *c* alkaline sucrose gradient Patterns of sedimentation of N4 DNA labelled continuously for the same period of time in rifampicin-pretreated cells *b*, neutral sucrose gradient, *d*, alkaline sucrose gradient The position to which marker ^{32}P-N4 DNA sediments is indicated by the arrows

polymerase The rifampicin-resistant mode of initiating Okazaki pieces[19], the replicative form of phage ΦX 174 (ref 20) and the λ*dv* plasmid dimer[8] could, in principle, be used by N4 DNA for priming the next round of replication More likely, one or both of the phage-related RNA-polymerising activities[13] may be involved in a drug-resistant transcription both at the origin and during elongation of N4 DNA Finally, an altogether different mechanism of DNA strand initiation and elongation cannot be ruled out on the basis of present data Isolation of a ribopolynucleotide covalently attached to a growing viral deoxypolynucleotide chain may place the mode of N4 DNA replication in a more usual frame and may render this system amenable to further investigation

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Poly(C) in animal viral RNAs

A TRACT of approximately 100 nucleotides, containing 80–90 cytidylic acid residues (poly(C)) has been found within the genome of encephalomyocarditis virus[1] (EMCV) We have sought to establish whether this unusual structural feature is also present in other animal picornaviruses, including those of human and veterinary importance, such as rhino-, foot-and-mouth disease and swine vesicular disease viruses The composition and extent of occurrence of poly(C) in a variety of picornaviruses might indicate whether it has a role in any of the processes which are common to all of these viruses, such as penetration, replication and assembly[2] Furthermore, if poly(C) is a virus-specific feature, it might prove feasible to devise antiviral chemotherapeutic strategies based upon its presence

Picornaviruses (family Picornaviridae) have been classified[3,4] into five subgroups on the basis of physicochemical evidence, namely the enteroviruses, cardioviruses, foot-and-mouth disease viruses (FMDV), and human and equine rhinoviruses We have examined representative viruses from four of these genera for the presence of poly(C) using methods similar to those previously employed with EMCV RNA, in which the poly(C) tract was detected as a very large T_1 RNase digestion product in polyacrylamide gels[1] All the cardioviruses and FMD viruses examined were found to possess a poly(C) region, we did not detect poly(C) in representatives of the entero- and human rhinoviruses

The viral RNAs were labelled by cultivating the viruses in medium containing ^{32}P-orthophosphate Figure 1 shows autoradiographs of polyacrylamide gel slabs in which the products of total T_1 RNase digestion of various ^{32}P-labelled RNAs isolated from the purified viruses were fractionated by electrophoresis. In those cases where very large products (≥50 nucleotides) were encountered, the fragments were analysed by further digestion with pancreatic RNase to determine whether they consisted largely of C residues (Table 1). The methods used in the further digestion of the C-rich tracts and in the fractionation and identification of their products are described in the legend to Table 1.

The RNAs of all examples of the cardioviruses and foot-and-mouth disease viruses studied yielded very large products on T_1 RNase digestion, containing mainly C (Table 1). In contrast, none of the six enteroviruses examined gave rise to any such C-rich products, although some seemed to contain A-rich tracts (ref. 5 and our unpublished observations). Similarly, the RNAs of three different rhinovirus strains yielded no large C-rich T_1 RNase digestion products. We have examined only one virus outside the picornavirus family, the togavirus, Semliki Forest virus[6]. T_1 RNase digestion of the virus 42S RNA and the virus-induced 42S and 26S RNAs gave rise to several A-rich products, but no C-rich oligonucleotides (I. Kennedy, A. P., C. N. and P. F., unpublished).

Table 1 Occurrence and analysis of C-rich fragments from total T_1 RNase digestion of various viral RNAs

Genus*	Virus	No. of analyses	Large C-rich T_1 RNase digestion products (Fig. 1)	Estimated size (nucleotides)	% C	Pancreatic RNase digestion products
Cardio	EMC	5	A_0	~100	80–90	1AAC, 1AC, 4U, 80–90C, 1G
	Mengo	3	M_0	~100	80–90	1AAC, 1AC, ~4U, 80–90C, 1G
			M_A	~150	> 75	mainly C
	Maus-Elberfeld	3	ME_0	~150	80–90	1AAAAC, 1AAU, 1AAC, ~2AU, ~4AC, ~6U, 90–110C, 1G(?)
Foot-and-mouth disease	A-61	3	A-61_0	~150	80–90	mainly C (some AC and U also present)
	O-V1	3	O-V_0	~150	80–90	$1A_3X$, 1AAC, 1AU, ~4AC, ~8U, 90–110C, 1G(?)
			O-V_B	⩾200	70–80	$1A_3X$, $1A_3C$, 1AAC, 5–10AC, 10–15U, ~150C, 1G(?)
	C-GC	2	C_A	⩾200	75–85	$1A_3X$, $1A_2C$, 1AU(?), ~10AC, ~17U, ~150C, 1G(?)
			C_B	⩾200	75–85	$1A_3X$, $1A_2C$, 1AU(?), ~10AC, ~17U, ~150C, 1G(?)
	C-997	1	not shown	~150	80–90	mainly C

* The RNAs of the following viruses did not give rise to large C-rich T_1 RNase oligonucleotides: the enteroviruses, polio (type 1, ts^+); Echo (type 12); coxsackie Faulkner; coxsackie 8068; bovine entero- and swine vesicular disease: the human rhinoviruses, types IA, 2 and 14: the togavirus, Semliki Forest.

The poly(C)-containing fragments, shown in column 4, were completely digested with pancreatic RNase, and the resultant oligonucleotides (column 7) were separated by electrophoresis on paper, quantitated and identified as previously described[1]. These compositions are calculated from different numbers of experiments (column 3) and enable the lengths of the fragments (column 5) to be estimated. These lengths are consistent with the mobilities of the fragments in 10% polyacrylamide relative to the electrophoretic front (Fig. 1). The C content of the large T_1 RNase-resistant fragments (column 6) is calculated from their pancreatic RNase digestion products. In those cases where there is uncertainty about whether G is molar, the yields of oligonucleotides are calculated assuming AAC is present once.

The large C-rich products arising from the cardioviruses and foot-and-mouth disease viruses shown in Table 1 fall into three size classes. The RNAs of EMCV and mengovirus each yielded products of about 100 nucleotides in length, containing at least 90% C (Fig. 1 and Table 1). It is clear that the nucleotide sequences of these viral RNAs are similar, since not only the Poly(C)-containing products A_0 and M_0, but also the smaller oligonucleotides A_1–A_3 (Fig. 1 and ref. 1) and M_1–M_3 (Fig. 1) gave rise to the same products upon subsequent pancreatic RNase digestion; however, two products were observed in mengovirus RNA which were not observed in EMCV RNA. The first was a large product, M_A, which occurred as a broad, possibly heterogeneous band in polyacrylamide gels (Fig. 1). It contained about 150 nucleotides as judged from its electrophoretic mobility, and from its intensity seemed submolar relative to M_0. Like M_0, the band M_A seemed to contain mainly C (Table 1). Its presence is noteworthy, since it is of similar size to the poly(C)-containing products arising from some of the other viruses examined (see below). The second T_1 RNase product, M_x, found in mengovirus but not in EMCV, also seemed rich in C (see Table 1). Its composition precludes it arising as a result of oversplitting of M_0.

Maus-Elberfeld virus (MEV) and the FMDV strains, A-61 and C-997, yielded C-rich products of about 150 nucleotides, as indicated both by their electrophoretic mobilities and their compositions. They each contained about 30–40 more C residues than the poly(C)-containing bands A_0 and M_0, respectively in EMCV and mengovirus RNA (see Table 1).

Finally, the RNAs of the FMDV strains O-V1 and C-GC gave rise to very large T_1 RNase digestion products containing at least 200 nucleotides. Strain C-GC yielded two large C-rich bands with very similar mobilities, apparently in equimolar amounts, which were barely separated in the 10% polyacrylamide gel. These bands (C_A and C_B) were almost identical in electrophoretic mobility to the large C-rich product (OV_B) from O-V1 RNA (Fig. 1). An additional C-rich band (OV_0) was encountered in O-V1 RNA with an electrophoretic mobility identical to that of the poly(C)-containing products from MEV and A-61 RNAs. The compositions of the poly(C) tracts set out in Table 1, however, do not preclude relationships between the larger digestion products of some of these viral RNAs, that is, the smaller products might have arisen by oversplitting of larger ones.

We are uncertain whether the C-rich products from MEV and the FMDV strains yielded molar amounts of G on subsequent pancreatic RNase digestion (Table 1). If these products lack G, they might be expected to arise from the 3′-terminus of the viral RNA. At this stage, however, our analyses of these products are insufficiently precise to enable us to discuss this possibility further.

The results described show that the presence of large tracts of poly(C) is not limited to EMCV RNA, but also occurs in the two other cardioviruses and three FMDV types examined. Similar tracts were not found in the RNAs of the other picornavirus genera. But if the poly(C) tract is involved in replication, for example as part of a recognition site for a viral replicase[7], it would be surprising if it is not conserved at least to some extent throughout the Picornaviridae, since the replication processes are believed to be similar for all these viruses[2]. The methods we have used here cannot resolve this question.

It is clear, however, that the grouping of the picornaviruses according to whether or not large C-rich oligonucleotides arise on digestion of their RNAs is in accord with a classification based on their physicochemical properties. For example, our results support the conclusion that the cardioviruses should be regarded as a genus distinct from the enteroviruses[3,8]. It is also of interest that EMCV and mengovirus, originally isolated from primates[9,10], gave identical poly(C) tracts, whereas the closely related Maus-Elberfeld virus (MEV), which was of murine

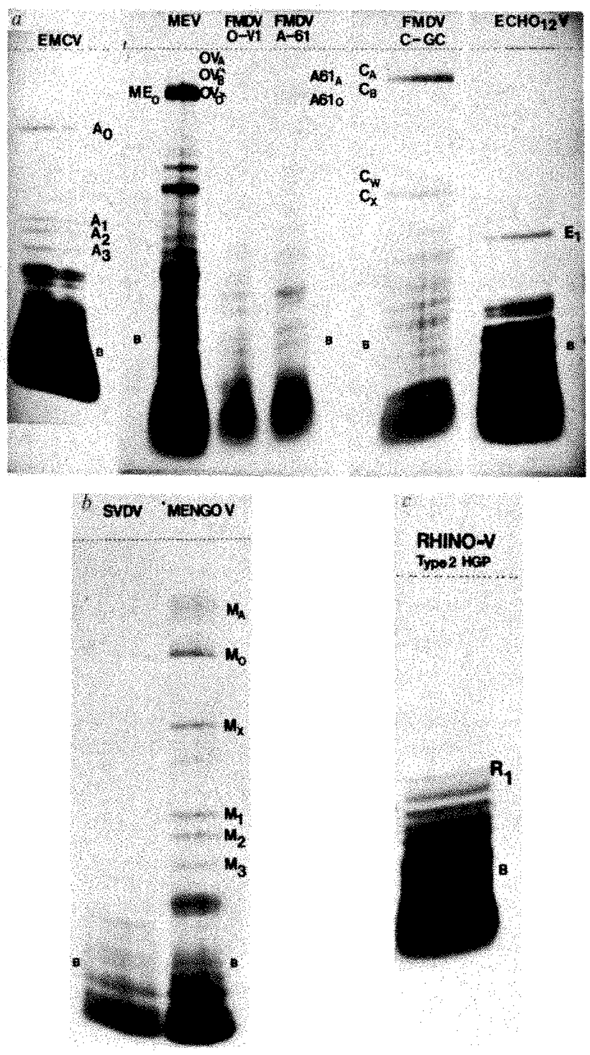

Fig. 1 The following viruses were grown in monolayers of the cell cultures indicated in parentheses in phosphate-free Earle's saline containing ^{32}P-orthophosphate: foot-and-mouth disease virus, strains O-V1, A-61, C-997 and C-GC (BHK-21); the cardioviruses, MEV and mengovirus (BHK-21); bovine enterovirus, VG-5-27 (BHK-21); swine vesicular disease virus, Italy 1/66 (IB-RS-2); coxsackie B5 virus, Faulkner strain and isolate 8068 (HeLa). The viruses were purified according to the method described for foot-and-mouth disease virus[12] and their RNAs extracted with phenol-SDS. Echo virus, type 12 (LLC-MK$_2$); poliovirus type 1, strain ts^{+} (HeLa S3); and the rhinovirus types 1A, 2 and 14 (L-132) were also grown in monolayers of cell cultures, except that they were purified and their RNAs extracted according to the method used for EMCV, given in ref. 1. Each sample of ^{32}P-labelled viral RNA (0.1×10^6–20×10^6 c.p.m. and containing 50–200 μg unlabelled carrier RNA) was dissolved in 0.02 M Tris-HCl *p*H 7.5; 0.002 M EDTA containing T$_1$ RNase (Sankyo, Japan) at an enzyme:substrate (w/w) ratio of 1:40. Digestion was complete in 1 h at 37° C. The resultant oligonucleotides were fractionated by electrophoresis in 10% polyacrylamide gel slabs as described previously (see Fig. 2 of ref. 1). The radioactive bands were located as shown by autoradiography, and excised using the autoradiograph as a guide. RNA was eluted electrophoretically from each numbered gel band on to DEAE-cellulose paper disks. Subsequent elution of the labelled fragments from the disks was performed using freshly prepared 30% triethylammonium carbonate, *p*H 10.0, as described by Sanger *et al*[13].

origin[11], gave a substantially longer tract of poly(C).

Thus, the occurrence of poly(C) regions in the picornaviruses seems to be genus specific. Furthermore, the lengths of the poly(C) tracts in the RNAs of individual members of the same genus, and even more significantly of subtypes of one serotype of an individual virus, can differ considerably. These findings emphasise the value of this type of analysis in the classification of viruses. Although clearly falling short of what could be achieved by complete nucleotide sequencing, the methods used here could lead to the elucidation of the variations in RNA primary structure which account for strain and serotype differences.

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Two functional states of the RNA polymerases in the rat hepatic nuclear and nucleolar fractions

IN eukaryotic cells, the DNA-dependent RNA polymerase is known to be bound to chromatin[1]. As it is known that chromatin is a restricted template[2,3], the question is whether all or only a fraction of the RNA polymerases bound to the chromatin is functionally active. The experiments reported here provide evidence that only a fraction of the RNA polymerases present in both nuclei and the nucleolar fraction is functionally active on chromatin.

It was noticed previously[4], when nuclei or nucleoli were treated *in vitro* with actinomycin D (10 μg ml^{-1}) to block the endogenous DNA-template function, that the RNA polymerase activity could be measured independently *in situ* in the same reaction vessel with a synthetic template, such as poly(dC) or poly(dA-dT). These synthetic templates are effective in directing RNA synthesis in the presence of actinomycin D, because they do not contain the deoxyguanosine moiety and hence do not react with the actinomycin D (ref. 5). As Fig. 1 shows when endogenous nuclear RNA synthesis was effectively blocked by actinomycin D, the incorporation of ^{14}C-UTP into RNA was an almost linear function of the concentration of poly(dA-dT). Fur-

Table 1 Functional independence of the engaged and free nuclear and nucleolar RNA polymerases

Incubation conditions	RNA polymerase activity pmol of labelled precursor incorporated per mg DNA ^{14}C–UTP	%	^{14}C–GTP	%
Nuclei	706	100	943	100
Nuclei + poly (dA–dT)	2,274	322	1,098	116
Nucleoli	995	100	1,535	100
Nucleoli + poly (dA–dT)	2,901	292	1,595	104
Nuclei	866	100	1,057	100
Nuclei + poly (dI–dC)	1,068	123	8,930	845
Nucleoli	6,599	100	11,686	100
Nucleoli + poly (dI–dC)	7,692	116	52,761	451

RNA polymerase activity was assayed as described in the legend to Fig. 1. When indicated, 25 μg poly(dA-dT) or 12.5 μg poly(dI-dC) (Boehringer Mannheim) was used as the exogenous template.

thermore, when nuclear or nucleolar suspensions were incubated *in vitro* in the absence of actinomycin D, the addition of poly(dA-dT) stimulated the incorporation of ^{14}C–UTP into RNA threefold (Table 1). If the RNA polymerases bound to chromatin were all functionally active toward the endogenous chromatin template, then all or part of the RNA polymerase would have to leave the chromatin and transcribe the poly(dA-dT) in order to account for increased ^{14}C–UTP incorporation into RNA after addition of the synthetic template. But, as the right hand columns of Table 1 show, when ^{14}C–GTP was used to monitor RNA synthesis, the addition of poly(dA-dT) did not enhance or antagonise incorporation. These results clearly indicate that the endogenous chromatin template retained all of the functionally active RNA polymerase (engaged form) for ^{14}C–GTP incorporation in the presence of the poly(dA-dT) synthetic template. Consequently, the RNA polymerase population that transcribed the poly(dA-dT) template had to be derived from a population of RNA polymerase, which was functionally inactive toward the endogenous chromatin template (free enzyme). The same conclusion was reached when poly(dI-dC) was used as the exogenous template. Where ^{14}C–GTP incorporation into RNA was stimulated several fold, again the endogenous template function, as indicated by ^{14}C–UTP incorporation data, was not significantly affected by the addition of the synthetic template (bottom part, Table 1).

Table 2 Effect of α-amanitin on the engaged and free nuclear RNA polymerase activity measured with endogenous and exogenous template

Incubation conditions	RNA polymerase activity pmol of ^{14}C–UMP incorporated per mg DNA	%	α-Amanitin sensitivity % Inhibition
Nuclei	790	100	30
+ α-Amanitin	551	70	
+ Actinomycin D poly(dA–dT)	931	118	30
+ Actinomycin D poly(dA–dT) α-amanitin	657	83	
+ Poly(dA–dT)	2579	326	52
+ Poly(dA–dT) α-amanitin	1244	157	
+ Actinomycin D	133	17	
+ Actinomycin D α-amanitin	105	13	
Solubilised nuclear enzyme			
+ Native calf thymus DNA	265		47
+ Native calf thymus DNA α-amanitin	141		

Assay conditions were the same as described in Fig. 1. When required, 2 μg α-amanitin (Henley, New York), 10 μg actinomycin D, and 25 μg poly(dA-dT), were added, singly or in combination, to the standard reaction mixture at time zero. Nuclear RNA polymerase was solubilised by the method of Roeder and Rutter[13]. Fraction 4 of the solubilised nuclear enzyme was used for the assay. Calf thymus DNA (25 μg) was used as template. Its activity was expressed as pmol of ^{14}C–UTP incorporated per mg protein.

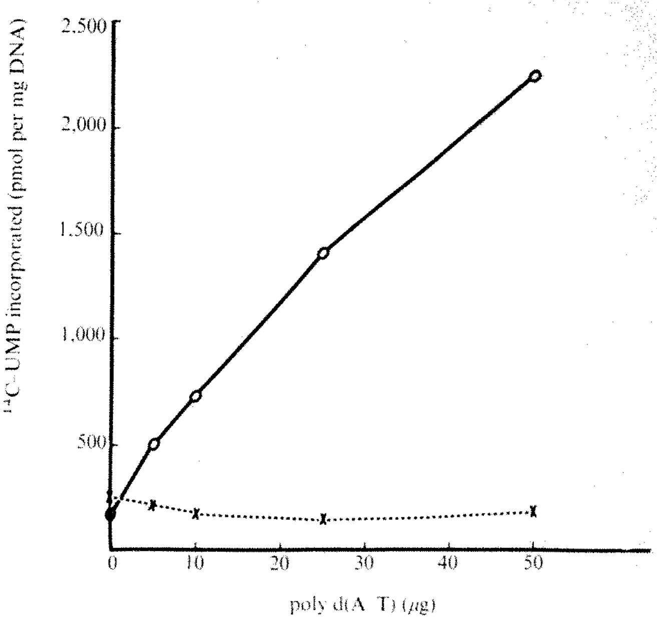

Fig. 1 Male Sprague-Dawley rats, about 200 g body weight, were used. Rat liver nuclei and nucleoli were isolated as described before[4]. RNA polymerase activity was assayed *in vitro* with a standard reaction mixture of 0.65–0.75 ml total volume containing (μmol): Tris-HCl (*p*H 7.9 at 23° C), 50; MnCl$_2$, 1; 2-mercaptoethanol, 14; (NH$_4$)$_2$SO$_4$, 40; and ATP, CTP, UTP and GTP, each, 0.1; with 0.1 μCi of either 2-^{14}C–UTP (Schwarz/Mann, specific activity 42 Ci mol^{-1}) or 8-^{14}C–GTP (Schwarz/Mann, specific activity 39 Ci mol^{-1}) as indicated. When required, 10 μg of actinomycin D (Nutritional Biochemicals) and an indicated amount of poly(dA-dT) (Miles) were also added to the incubation mixture at time zero. The reaction was initiated by the addition of 0.1 ml nuclear or nucleolar suspension in 0.34 M sucrose derived from 0.1 and 0.6 g of liver, respectively, and incubated for 10 min at 37° C with shaking. The reaction was stopped by transfer of the reaction tubes to chipped ice, followed by immediate addition of 5 ml of cold 10% trichloroacetic acid containing 1% sodium pyrophosphate. The acid-insoluble material was collected on Whatman GF/C filters and washed seven times with 5 ml of 5% cold trichloroacetic acid containing 1% sodium pyrophosphate, and once with 5 ml of 60% ethanol. The filters were air dried in liquid scintillation vials and counted in 10 ml of Bray's solution[10]. The specific activity of RNA polymerase was expressed as pmol of labelled ^{14}C–UMP or ^{14}C–GMP incorporated per mg DNA. DNA concentrations were determined as described by Burton[11]. Protein was measured by the method of Lowry *et al.*[12] with bovine serum albumin as standard. Nuclei + actinomycin D (×); nuclei + actinomycin D + poly(dA-dT) (○).

Several species of RNA polymerases are known to exist in eukaryotic nuclei[6]. Since α-amanitin specifically inhibits nucleoplasmic RNA polymerase II (refs 7–9), it was decided to use it as a probe to determine whether the chromatin bound RNA polymerases in both the 'engaged' and 'free' states represent the same enzyme. The results presented in Table 2 show that both the 'engaged' and 'free' nuclear RNA polymerases were similarly inhibited by α-amanitin. For example, the activity of the 'engaged' enzyme on the

Table 3 Effect of α-amanitin on the engaged and free nucleolar RNA polymerase activity measured with endogenous and exogenous template

Incubation conditions	RNA polymerase activity pmol of ^{14}C–UMP incorporated per mg DNA	%	α-Amanitin sensitivity % Inhibition
Nucleoli	1,434	100	
+ α-Amanitin	1,289	90	10
+ Actinomycin D poly(dA–dT)	2,411	168	
+ Actinomycin D poly(dA–dT) α-amanitin	2,534	177	0
+ Poly(dA–dT)	4,614	322	
+ Poly(dA–dT) α-amanitin	3,928	274	15
+ Actinomycin D	0	0	
+ Actinomycin D α-amanitin	0	0	

Conditions were the same as described in the legend to Table 2.

endogenous chromatin template was inhibited 30% by α-amanitin, whereas the 'free' polymerase, which utilised the exogenous poly(dA-dT) template, was also inhibited 30% under conditions where the endogenous chromatin template activity was inhibited by actinomycin D. In the absence of actinomycin D, the inhibition was 52%. This level of inhibition agrees very well with the inhibition of the solubilised RNA polymerase whch was 47% when calf thymus DNA was used as template.

Table 3 presents data which indicate that this toxin had no effect on both the 'engaged' and 'free' nucleolar RNA polymerases, as has been reported[7-9]. These data suggest that the 'engaged' and 'free' forms of RNA polymerase in nuclei most probably represent the same enzyme, and similarly the 'engaged' and 'free' forms present in the nucleolus also are derived from a single population of RNA polymerase molecules.

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Location of human satellite DNAs on the Y chromosome

THE technique of *in situ* hybridisation of radioactively labelled DNA or RNA to the denatured DNA in metaphase chromosomes provides a powerful and popular method for identifying the chromosomal location of specific repeated DNA sequences[1,2]. In man, this technique has been used to map the ribosomal cistrons (ref. 3 and H.J.E., M. L. Pardue, and R.A.B., in preparation) and to examine the chromosomal location of three of the four known satellite DNAs[4-8]. Satellite II (ref. 6) was found in the centromeric heterochromatic regions (C band) of chromosome 1 and in the C bands of one C group (chromosome 9) and one E group chromosome pair (tentatively identified as chromosome 16). Satellite III (ref. 8) was found mainly in the C band of chromosome 9, with some in the centromere/short arm regions of the D and G group chromosomes, and possibly in the C bands of chromosomes 1 and 16. The satellite DNA investigated by Saunders *et al.*[7] (which is probably identical to satellite IV of Corneo *et al.*[5]) was only positively identified in the C band of chromosome 9, although other regions of hybridisation were noted. In all these studies on satellite DNAs, the autoradiographic observations were limited because no general chromosome identification was possible. Thus, only major sites of hybridisation could be detected and in some instances the locations of these sites were in doubt.

We have now investigated the chromosome location of the four human satellite DNAs by *in situ* hybridisation using dual karyotype analysis (ref. 9 and J.R.G., R.A.B., R. P. Clayton and H.J.E., in preparaton) (see legend to Fig. 1). This involves quinacrine fluorescence and karyotype analysis of the chromosomes, followed by *in situ* hybridisation and comparison of the autoradiographs with the corresponding fluorescence karyotype. The method allows unequivocal identification of each site

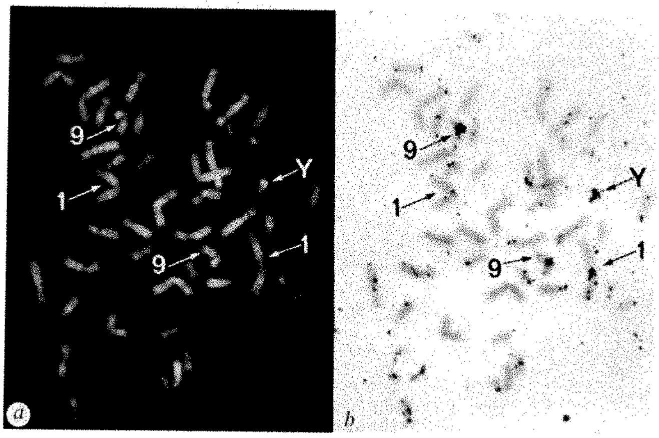

Fig. 1 DNA was prepared from placentae of male babies by the method of Marmur[11]. Satellite DNAs were isolated by centrifugation in Ag^+–Cs_2SO_4 or Hg^{2+}–Cs_2SO_4 density gradients[4,5] and buoyant densities and purity checked in neutral CsCl gradients. ^{3}H-labelled cRNAs were prepared *in situ* hybridisation performed as described elsewhere (J.R.G., R.A.B., R. P. Clayton and H.J.E., in preparation). The cRNAs were hybridised to blood lymphocyte chromosome preparations from various normal, and chromosomally abnormal males and females. Chromosomes were first stained with quinacrine and selected well spread metaphase cells showing good Q banding under fluorescence photographed. Following removal of the stain by washing, hybridisation, autoradiography and staining with Giemsa or Crystal Violet, these cells were again located, re-photographed and dual karyotypes constructed (ref. 9 and J.R.G., R.A.B., R. P. Clayton and H.J.E., in preparation). *a*, Fluorescence photograph of quinacrine stained chromosomes before *in situ* hybridisation. *b*, Giemsa stained autoradiograph of same cell after hybridisation with cRNA prepared from DNA satellite II. Note the concentration of grains over both chromosome 9 and the Y chromosome and that the differences in grain count between homologous chromosomes of pairs 1 and 9, parallel differences in the sizes of their respective C bands.

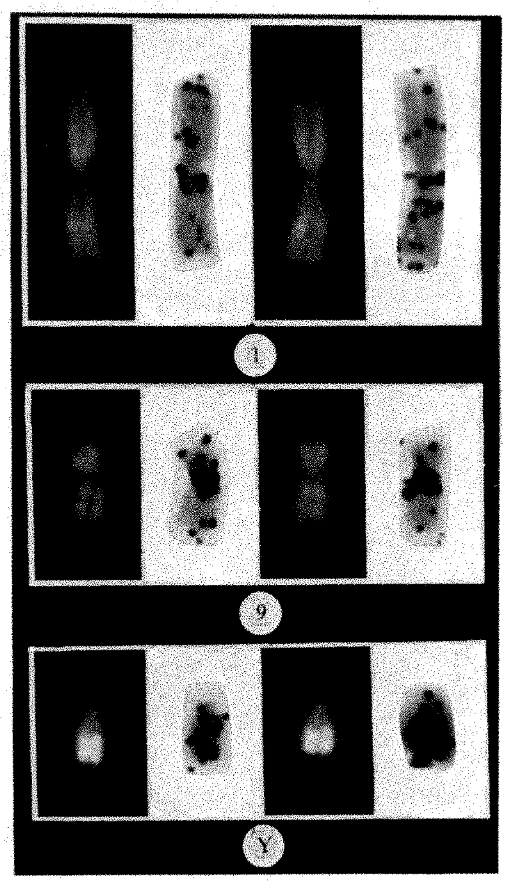

Fig. 2 Partial dual karyotype of chromosomes (1, 9 and Y) prepared from a blood lymphocyte of a 47,XYY male and hybridised with cRNA prepared from DNA satellite IV. All methods were as described in the legend to Fig. 1.

of hybridisation. Full details of these methods and results will be published elsewhere (J.R.G., A.R.M., R.A.B., R. P. Clayton, and H.J.E., in preparation), but we would like to comment here on one important aspect of our findings.

Although our results differ in several minor respects from those previously published, one major location of satellites II, III and IV seems to have been completely overlooked. We find that a substantial portion of all three of these satellites, and also of satellite I, is located in the human Y chromosome, in particular in the brightly fluorescent region of the long arm (Fig. 1)—the region that stains intensely with Giemsa with the C-banding technique. This specific hybridisation to the Y chromosome is a consistent finding, made in numerous experiments using cells from normal males and from males with a 47,XYY chromosome constitution. In the latter case, hybridisation is found on both Y chromosomes and an example is illustrated in Fig. 2.

The apparent discrepancy between our finding and those previously published cannot be accounted for by errors in observation, since the Y chromosome can be identified without recourse to fluorescence analysis, and the amount of cRNA hybridised is large. Saunders *et al.*[7] hybridised cRNA to cultured fibroblast metaphase cells and there is no mention of the Y chromosome or the sex of the donor(s) of these cells. The cRNAs from satellites II and III, however, were hybridised to cells containing a Y chromosome, but no specific hybridisation to the Y was noted[6,8]. The difference is unlikely to be in the origins of the template DNAs used by the different groups, but may be a consequence of differences in techniques used in the hybridisation process.

We have found that the Y chromosome contains a considerable proportion of repetitious DNA, and that some of this is in a satellite-free fraction (J.R.G., R.A.B., R. P. Clayton, and H.J.E.; J.R.G. and A.R.M., in preparation). The present studies show that the human Y chromosome also contains repetitious sequences from all four of the human satellite DNA fractions. Previous cytochemical results have suggested that the human Y chromosome may contain highly repetitious DNA[10], but this is the first direct demonstration of this fact.

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Murine leukaemia virus RNA transcription from chromatin of normal and infected BALB/c spleen

STUDIES on Rous sarcoma virus (RSV), murine leukaemia virus (MuLV) and mammary tumour virus (MTV) systems have shown that at least parts of viral genomes are present in multiple copies in the natural host DNA. RSV-specific nucleotide sequences have been detected in various chick cells, including Rous sarcomas and embryos with or without RSV gs antigens and chick helper factor[1,2]. Similarly, normal and virus-transformed mouse and rat cells contained equivalent amounts of MuLV-specific DNA sequences[3]. MTV-specific DNA genomes have been detected in equal numbers in mouse strains with both high and low incidences of mammary tumours[4]. In addition, induction of leukaemia viruses from apparently uninfected mouse and chicken cells indicates that complete, 'endogenous' viral genomes are present in these cells[5-8]. But the integration of 'exogenous' viral genomes, upon infection with an oncogenic RNA virus, cannot be proved in an homologous system because of the lack of specific markers which could distinguish between exogenous and endogenous MuLV genes. Actual integration was demonstrated in the case of some heterologous systems, upon infection of duck and of mouse cells with RSV[9].

Evidence exists that partial transcription of MuLV genomes occurs in a virus-free cell line, but to a lesser extent than in some of its transformed derivatives[10]. A similar observation was reported in the case of MTV-RNA transcripts in tumours, lactating mammary glands, spleens and livers from high and low incidence mouse strains[11]. More direct evidence for transcription of integrated viral genes was obtained by studying DNA-containing tumour virus systems. Polycistronic RNA molecules containing both cellular and viral base sequences were reported in adenovirus and in simian virus 40 (SV40) transformed cells[12,13]. Moreover, Astrin[14] and Shih *et al.*[15] demonstrated that chromatin isolated from SV40-transformed mouse cells can synthesise SV40 RNA transcripts *in vitro*. Here

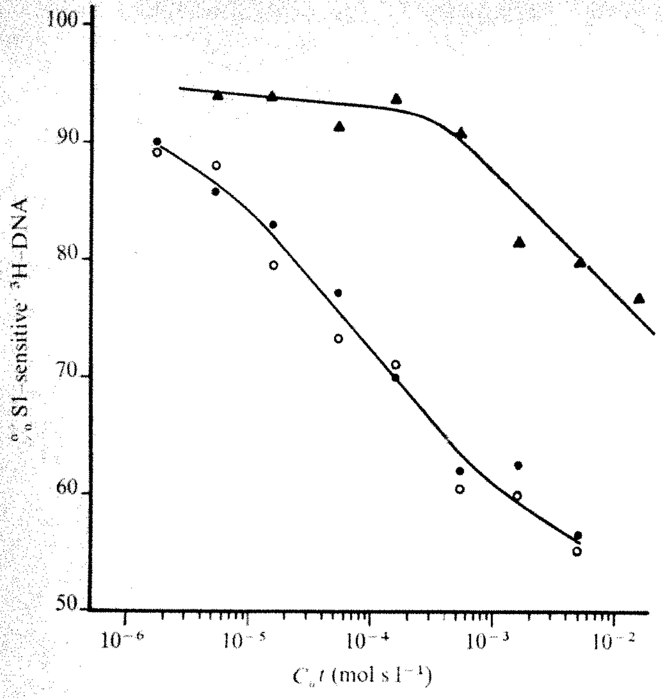

Fig. 1 Annealing curves of RLV–^{3}H–DNA in the presence of 15 mg ml^{-1} of either salmon sperm DNA (▲), normal BALB/c spleen DNA (●), or RLV-infected BALB/c spleen DNA (○). RLV was obtained from Electronucleonics. The virus (approximately 1 mg protein) was incubated for 18 h at 37° C in a 8 ml standard reaction mixture and the ^{3}H–DNA product was purified as described[21]. Non-radioactive DNA was extracted from isolated spleen nuclei of normal and RLV-infected BALB/c mice. The extraction procedure was essentially that of Marmur[22]. It is included two 30 min incubations at 37° C in the presence of 100 μg ml^{-1} RNase and two 30 min incubations at 37° C in the presence of 400 μg ml^{-1} pronase. Salmon sperm DNA (Sigma) was treated with RNase and pronase, and deproteinised before use. DNA fragments were prepared by sonication (Mullard sonifier) and their molecular weight, as determined by analytical ultracentrifugation (Beckman model E), ranged from 130,000 to 160,000 daltons. The DNA samples to be annealed were denatured for 15 min at 100° C in 30 mM Tris, *p*H 7.6. After rapid chilling at 0° C, NaCl was added to a final concentration of 0.6 M. Fifteen microlitre duplicate samples, containing each 4,500 d.p.m. of viral ^{3}H–DNA (Sigma). One of the duplicate samples contained 80 ng 67° C in sealed glass capillaries (Drummond microcaps) for periods of time ranging from 1 min to 6 d. After incubation, the content of each capillary was chilled and poured into 0.6 ml 30 mM sodium acetate, *p*H 4.6, 50 mM NaCl, 1 mM ZnSO$_4$, 5% glycerol and 50 μg ml^{-1} native thymus DNA (Sigma). One of the duplicate samples contained 80 ng ml^{-1} (8 activity units ml^{-1}) of *Aspergillus oryzae* SI-nuclease (prepared according to ref. 23). The mixtures were incubated for 15 min at 45° C, chilled, precipitated in the cold by 1.2 ml 10% perchloric acid (PCA) and filtered through Whatman GF C filters. The filters were washed with 10 ml 5% PCA, dried under a heat lamp and counted in a Packard scintillation counter. The percentage of reannealed ^{3}H–DNA refers to the ratio of SI-nuclease-resistant radioactivity to the total radioactivity in the samples that were not submitted to SI-nuclease treatment. No loss of total PCA-precipitable radioactivity was observed after incubation periods up to 6 d. The results presented in the graphs were not corrected for the presence of nonreannealing ^{3}H–DNA present in the viral product.

we report that MuLV-DNA copies are present in equal amounts in spleen DNA from both normal mice and mice infected with Rauscher leukaemia virus (RLV), but that the viral genomes are expressed to a much larger extent in leukaemia mouse spleen chromatin than in normal spleen chromatin.

RLV-infected BALB/c mice were killed 15 d after virus inoculation. Infected and non-infected spleens were both processed in the same way for DNA extraction, as described in the legend to Fig. 1. The unlabelled cellular DNAs were tested for virus-specific nucleotide sequences by their capacity to accelerate the reassociation kinetics of labelled DNA, synthesised *in vitro* by partially disrupted RLV virions. The apparent reduction in the C_0t values (product of initial concentration of DNA, in mol of nucleotide per l, and time (s)), showed that the reassociation rate of the ^{3}H-DNA product was, in the presence of either infected or uninfected BALB/c spleen DNA, increased about 300-fold over the rate observed in the presence of salmon sperm DNA (Fig. 1). At these concentrations of cellular DNA and viral ^{3}H-DNA product, this degree of acceleration is consistent with the presence of about 40 copies of viral sequences per diploid mouse genome. This result is in good agreement with those of Gelb *et al.*[3], who reported 50 leukaemia virus-specific DNA copies per diploid BALB/3T3 cell.

Subsequently we determined the level of *in vivo* expres-

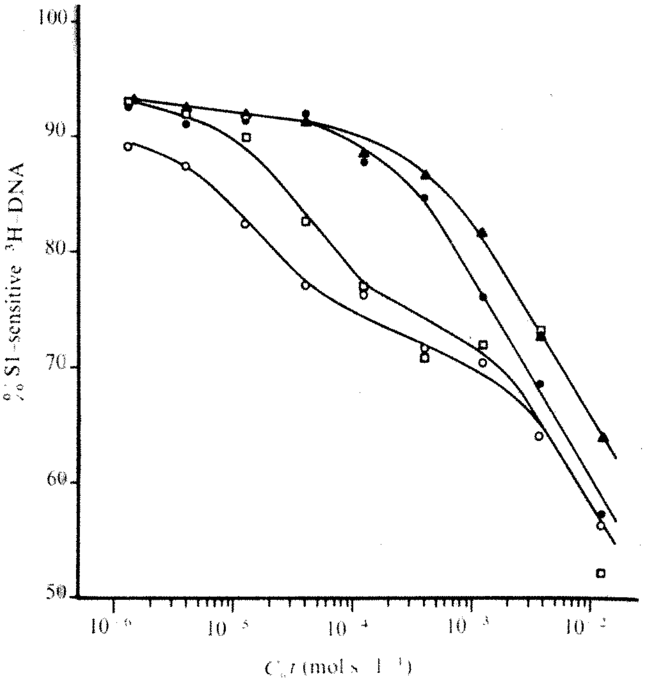

Fig. 2 Annealing curves of RLV–^{3}H–DNA in the presence of 1.4 μg ml^{-1} 70S RLV-RNA (□), 200 μg ml^{-1} endogenous RNA from normal BALB/c spleen chromatin (●), 600 μg ml^{-1} endogenous RNA from RLV-infected BALB/c spleen chromatin (○), or no chromatin nor viral RNA (▲). High molecular weight (70S) RLV-RNA was purified from infected plasma virions according to ref. 24. Chromatin was purified from spleens as described[25], except that the ultimate centrifugation step was omitted to avoid the possible loss of a preferential DNA fraction. It was suspended at a concentration of 150 μg DNA ml^{-1} in 50 mM Tris, *p*H 8.0. NaCl and SDS were added to a final concentration of 0.4 M and 1%, respectively. Deproteinisation was performed on 100 ml volumes (15 mg chromatin DNA) at room temperature by two extractions with an equal volume of phenol-cresol (7:1) and one extraction with chloroform-isoamyl alcohol (24:1). Nucleic acids were ethanol-precipitated, dissolved in 6 ml 10 mM Tris, *p*H 7.6, 0.1 M NaCl and 5 mM MgCl$_2$, and incubated for 60 min at 37° C after addition of 250 μg DNase I (Sigma). Deproteinisation in the presence of SDS and NaCl, and ethanol precipitation were repeated as before. The resulting precipitate was suspended in a minimal volume of 10 mM Tris, *p*H 7.6, and the sample was passed over a 1.6 cm × 100 cm Sephadex-G-50 (medium) column equilibrated with 10 mM Tris, *p*H 7.6. The RNA eluting in the excluded volume was ethanol-precipitated and used for annealing experiments with viral ^{3}H-DNA. The annealing reactions were performed in 20 μl duplicate samples containing 4,500 d.p.m. RLV-^{3}H-RNA, 200 μg yeast RNA (Sigma) and RNA to be tested. The procedure was the same as for annealing in the presence of DNA, except that the SI-nuclease reaction mixtures contained 150 μg ml^{-1} calf thymus DNA and that precipitation was performed with TCA (trichloroacetic acid) instead of PCA.

sion of the viral DNA genomes in chromatin isolated from normal and RLV-infected BALB/c spleens Chromatin from both normal and infected spleens was purified and processed for RNA extraction as described in legend to Fig 2 RLV-^{3}H-DNA was again used as a probe for virus-specific nucleotide sequences in chromatin RNA High molecular weight (70S) RLV-RNA was used as a viral RNA standard Leukaemic spleen chromatin RNA accelerated the reassociation kinetics of the probe ^{3}H-DNA to a much higher degree than did uninfected spleen chromatin RNA (Fig 2), but only over a limited *C₀t* range The latter observation might be explained by the possible presence of nonviral nucleotide sequences in the ^{3}H-DNA product The acceleration rates, when compared with the acceleration effect of the 70S viral RNA, are consistent with the presence of 0 7 and 0 06% viral sequence in the RNA population of leukaemic and normal spleen chromatin, respectively

Endogenous chromatin RNA might consist mainly, if not exclusively, of newly *in vivo* synthesised molecules If so, *in vitro* transcription of chromatin by RNA polymerase would not result in a measurable enrichment of the total RNA population in virus-specific sequences On the contrary, chromatin might contain appreciable amounts of associated RNA, considered as a distinct, structural and/or regulatory component, which does not seem very likely[16–18] In the latter case, the *in vitro* RNA transcripts would contain a higher proportion of viral sequences than the endogenous chromatin RNA Our experiments favoured the first hypothesis we incubated infected and uninfected spleen chromatin in the presence of all four unlabelled ribonucleoside triphosphates Bacterial enzyme was used because, besides the fact that it is easier to prepare in large amounts, it displays a good fidelity in chromatin transcription[14] The incubation resulted in a fivefold increase of the RNA content in the reaction medium This RNA accelerated the reassociation of the probe ^{3}H-DNA to the same degree as did the corresponding, endogenous chromatin RNA In the case of RNA from leukaemic spleen chromatin, the acceleration occurred throughout the observed *C₀t* values, in contrast with the effect of either 70S RLV-RNA or endogenous leukaemic spleen RNA This observation suggests that *in vitro* chromatin transcription yields RNA sequences that are not present, either in the endogenous chromatin RNA population, or in the high molecular weight RLV-RNA These additional sequences are, however, complementary to the ^{3}H-DNA probe Hence the probe should be considered as only partially virus specific

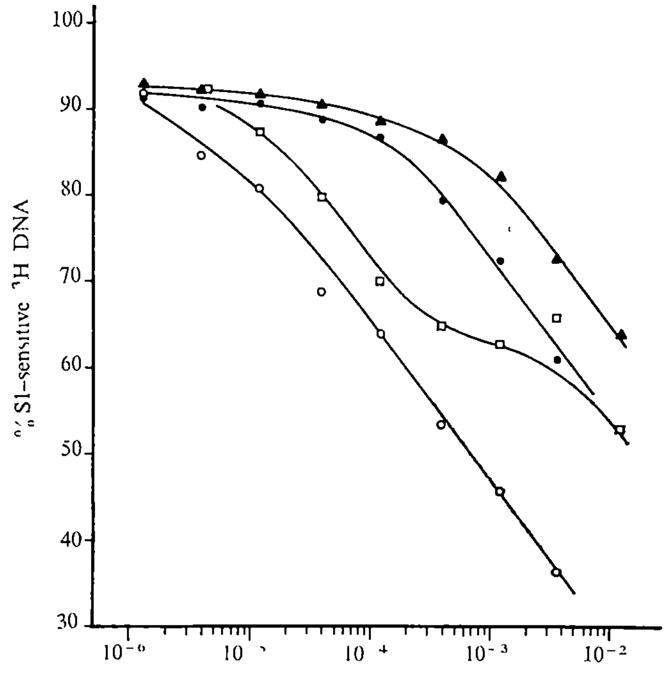

Fig 3 Annealing curves of RLV-^{3}H-DNA in the presence of 1 4 μg ml^{-1} 70S RLV-RNA (□), of 1 1 mg ml^{-1} *in vitro* RNA transcripts from normal BALB/c spleen chromatin (●), of 1 3 mg ml^{-1} *in vitro* RNA transcripts from RLV-infected BALB/c spleen chromatin (○), or neither chromatin nor viral RNA (▲) *E coli* RNA polymerase was purified according to Burgess[26], using the alternate method of gel filtrations on Bio Gel A-5m and Bio Gel A-1 5 m columns *In vitro* synthesis of RNA was performed as follows a 100 ml reaction mixture, containing 50 mM Tris, *p*H 8 0, 100 mM NaCl, 4 mM MgCl$_2$, 1 mM MnCl$_2$, 0 1 mM DTT, 0 2 mM of each unlabelled ATP, CTP, GTP and UTP, 150 μg ml^{-1} DNA in the form of chromatin, and 150 μg ml^{-1} RNA polymerase, was incubated for 45 min at 37° C The reaction was terminated by addition of NaCl and SDS to a final concentration of 0 4 M and 1%, respectively All subsequent operations were performed as described in legend to fig 2

This work clearly demonstrates that endogenous chromatin RNA from RLV-infected spleens, as well as *in vitro* RNA transcripts from the same spleen chromatin, contains very significantly more virus-specific sequences than the corresponding RNAs from the non-infected spleen chromatin Thus, our experiments provide direct evidence that the viral genomes, present in the natural host DNA, are largely expressed in RLV-infected spleen A much lesser degree of expression occurs in the normal tissue

Whether the transcription from leukaemic spleen chromatin occurs at the level of pre-existing or of newly integrated viral genes cannot be discussed here because of the lack of specific markers Finally, we feel that our experimental approach is more direct than those consisting of the detection of viral sequences in cytoplasmic[11,19], polyribosomal[20] or even nuclear[11] RNA populations

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Detection of cell-dependent cytotoxic antibody to cells infected with herpes simplex virus

The killing or damage of virus-infected cells by immune processes may play a significant role either in host defence or in the production of pathological lesions during virus infections[1] These immune processes are believed to be directed at virus-induced antigens on the membranes of infected cells On the basis of *in vitro* experiments, several mechanisms have been proposed as possibly operative *in vivo* These include the cytolytic action of antibody and complement[2,3], the interaction of antibody, complement and leukocytes[4], and the destruction of infected cells by immune lymphocytes[5–7] Another mechanism described in non-viral systems is the cytotoxicity demonstrated by the synergistic effect of antibody and nonimmune effector cells[8–10] The following experiments demonstrate a similar process operative with viruses in which heat-inactivated immune serum augments the damage of herpes simplex virus-infected cells *in vitro* by human mononuclear cells

Blood from human donors, whose sera contained or lacked neutralising antibodies[11] to herpes simplex virus type 1 (HSV-1), was used Mononuclear cells (MC) were purified from heparinised blood by centrifugation on a layer of Ficoll-Hypaque[12], washed four times in Hanks balanced salt solution (BSS), counted and diluted in Eagle's growth medium containing 10% inactivated foetal calf serum (EGM) Ninety-five per cent of the cells were mononuclear, about 90% of which were lymphocytes, cell viability exceeded 90% All human or rabbit sera to be tested were heat inactivated at 56° C for 30 min The γ-globulin and purified human IgG described below were not heat inactivated

Table 1 Absorption of augmenting factor by HSV-1-infected BHK-21 cells

Added to MC	Absorption with	% cytotoxicity	% augmentation
PHI 1/500	Not absorbed	36 1±1 8	22 8
PHI 1/500	HSV-1-infected BHK	13 4±1 2	0 1
PHI 1/500	Control BHK	32 1±1 7	18 8
Culture medium	- - -	13 3±2 8	0 0

2 0 ml of a 1/50 dilution of pooled HSV-1 immune serum (PHI) in BSS was mixed with an 0 3 ml pellet of either BHK-21 cells previously infected with HSV-1 or control BHK cells The mixture was agitated for 120 min at room temperature and then centrifuged The resulting supernatant was absorbed with a second pellet of infected or control cells for 24 h at 4° C and centrifuged before testing for the presence of augmenting factor Augmentation is defined as the difference between MC+test substance and the MC control (line 4)

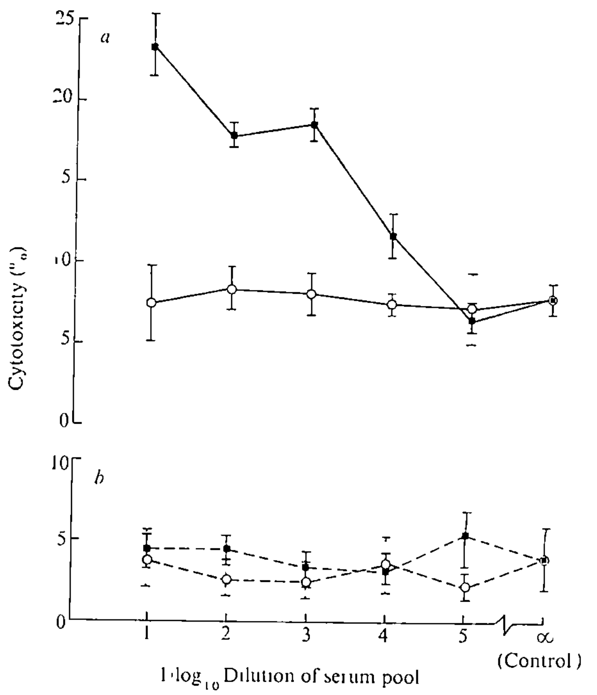

Fig 1 Augmentation of cell mediated cytotoxicity by antibody-positive pooled human sera MC plus dilutions of the seropositive pool (■) are compared with MC plus dilutions of the seronegative pool (○) 5 × 10^5 human MC (obtained from a seropositive donor) with or without positive or negative serum were added per well An effector target cell ratio of 50 1 was found to be optimal in separate experiments The positive and negative pools comprised sera taken from five positive and negative donors, respectively *a*, HSV-1 infected HEp 2 target cells *b*, uninfected HEp 2 target cells

HEp-2 cells (3 × 10^6) were exposed simultaneously to 100 μCi ^{51}Cr and 3 × 10^6 plaque-forming units (PFU) of the Heard strain of HSV-1 in a final volume of 1 0 ml of Eagle's maintenance medium containing 2% inactivated foetal calf serum (EMM) Cells were incubated for 150 min at 37° C and washed four times, 1 × 10^4 cells diluted in EMM were then added to each well of a Falcon Micro Test II plate Control HEp-2 cells were treated in a similar fashion except that no virus was added The sealed plates were incubated at 37° C in a CO_2 incubator and used 24–32 h later when approximately 50% of the infected cells showed cytopathic effects (CPE) At this time, the EMM was removed from each well by aspiration with a Pasteur pipette, and discarded MC, sera or EGM, alone or in combination, in a total volume of 0 25 ml, were added at 37° C for another 8–12 h, unless stated otherwise (see below and Fig 2) The bathing medium was then aspirated carefully with a Pasteur pipette, the monolayer lysed with 1 M NaOH, and the medium and cell lysate transferred to tubes for counting separately in a Nuclear–Chicago gamma counter All determinations were performed in quadruplicate Cytotoxicity was defined, following the practice of Holm and Perlmann[13], as the percentage of ^{51}Cr release observed with lymphocytes and other immune factors less the percentage ^{51}Cr released spontaneously from HSV-1-infected or control HEp-2 cells

During the 8–12 h incubation after the addition of MC and serum, the CPE in the infected cultures increased to involve approximately 90% of the cells Nevertheless, most cells remained attached to the well surface for at least another 12 h Assessment of cell viability after the 12 h incubation revealed that only ~15% of the attached cells stained with Erythrocin B as compared with ~5% of the cells in the control cultures During this incubation, infected and control HEp-2 cells showed 17–37% (mean 29)

Table 2 Titration of human commercial γ-globulin and purified IgG

Added to MC	Dilution	Infected cells % cytotoxicity	Infected cells % augmentation	Control cells % cytotoxicity	Control cells % augmentation
γ-globulin	1/500	45.1±2.9†	26.2	12.3±1.2	−2.3
	1/5,000	43.0±2.4†	24.1	16.2±2.3	1.6
	1/50,000	36.0±0.8†	17.1	15.7±3.0	1.1
	1/500,000	21.5±2.2	2.6	19.4±2.8	4.8
	1/5,000,000	22.7±1.9	3.8	17.4±2.4	2.8
	1/50 000,000	20.8±2.1	1.9	14.4±2.2	−0.2
Culture medium	- - - -	18.9±3.5	0.0	14.6±4.5	0.0
IgG	1/1,500*	34.2±4.1†	13.7	12.3±1.3	−0.7
	1/15,000*	25.9±2.5‡	5.4	13.9±0.6	0.9
	1/150,000*	22.9±2.4	2.4	12.6±1.7	−0.4
	1/1,500,000*	21.2±1.2	0.7	12.8±1.3	−0.2
Culture medium	- - - -	20.5±2.8	0.0	13.0±1.0	0.0

Hyland γ-globulin (TA No 0442R011A7), dialysed to remove preservative, and lyophilised human IgG (Mann, V-3122) were tested. In addition to IgG, the γ-globulin contained 1.7 mg ml^{-1} of IgM as assessed by radial immunodiffusion[14]. At 4 mg ml^{-1}, the IgG material gave only the typical arc of IgG on immunoelectrophoresis against rabbit anti-human serum, it contained no IgM by radial immunodiffusion (sensitivity < 8 μg ml^{-1} IgM). Neither γ-globulin nor IgG in the absence of MC was more cytotoxic than culture medium for the infected target cells.

* Dilution is expressed in relation to the usual IgG concentration of pooled normal adult sera of about 11.5 mg ml^{-1}

† Significantly different from culture medium control, $P < 0.001$

‡ $P < 0.01$

§ $P < 0.05$

and 16–30% (mean 22) spontaneous ^{51}Cr release, respectively, within a given experiment, infected cells showed 5–8% more background ^{51}Cr release than the control cells. Centrifugation (1,500 r.p.m. for 15 min) of the aspirated bathing medium taken from the infected cultures, however, revealed that about 10% of the total c.p.m. were sedimentable, as compared with 3% for control cultures. Therefore the increased ^{51}Cr release of the HSV-1-infected cells appeared to reflect an increment in the relatively small number of loose or dead cells shed into the aspirated medium rather than an increased loss of ^{51}Cr-labelled cellular macromolecules.

Figure 1 presents data typical of four experiments. Cell-mediated cytotoxicity was augmented by the presence of pooled HSV-1 antibody-positive human serum but not by pooled serum with no detectable HSV-1 antibodies. The increments in cytotoxicity were apparent at dilutions 1/10 to 1/10,000 and were statistically significant at $P < 0.001$ (Student's *t* test). The serum pools alone (without MC) had no effect on ^{51}Cr release. The increment due to the addition of serum was reproducible but varied in magnitude from experiment to experiment (range 8–30%). In separate experiments, rendering the aspirated bathing medium cell free by centrifugation did not reduce the difference in leakage observed between infected HEp-2 cells incubated with MC and positive serum and infected HEp-2 cells incubated with MC alone. This implies that the augmenting effect of positive serum was on ^{51}Cr release *per se* and not on the sloughing of cells into the culture medium. There was also a small but consistent cytotoxic effect of MC alone on the infected as compared with the control target cells (Fig. 1). The results from additional experiments showing that this effect can be obtained with MC from either seropositive or seronegative donors, however, would indicate that cell-mediated cytotoxicity conveyed by sensitised effector cells alone is not involved.

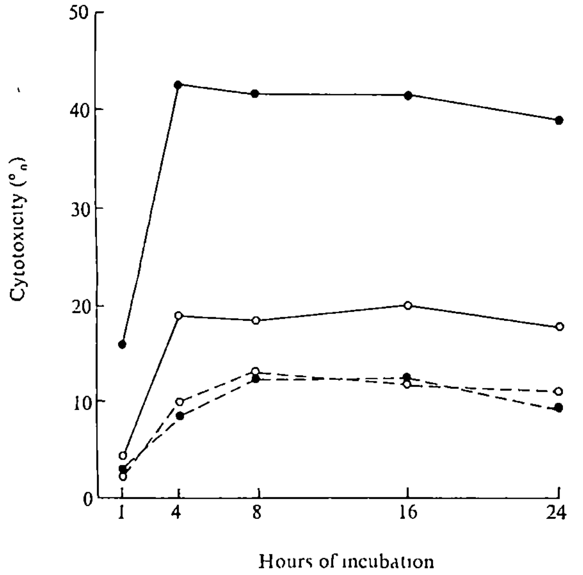

Fig. 2 Effect of time of incubation on augmented cytotoxicity. ———, HSV-1-infected cells; – – – –, control cells; ●, mononuclear cells (from a seropositive donor) (5 × 10^5 per well and 1/500 antibody-positive serum; ○, mononuclear cells alone.

At a 1/500 dilution of the positive serum, a dilution at which no cytolytic (complement-dependent) antibody could be detected, the augmented cytotoxicity was readily apparent 1 h after the addition of the MC and was maximal at 4 h (Fig. 2). In subsequent study of this phenomenon, an 8–12 h incubation was used.

Nine human immune sera and eight nonimmune sera were tested individually at a 1/500 dilution for augmentation of cytotoxicity. All sera containing HSV-1 neutralising antibody augmented cytotoxicity, in contrast the sera with no detectable HSV-1 antibody failed to do so. In addition, serum from an HSV-1-immunised rabbit showed augmentation, whereas nonimmune rabbit serum did not. The ten immune sera showed augmentation of 11.0 ± 1.8% compared with 0.5 ± 1.3% for the nonimmune sera ($P < 0.001$).

The augmenting factor could be removed by absorption of antibody-positive human serum with HSV-1-infected BHK-21 cells but not by uninfected BHK-21 (Table 1). It was also present in high titre both in pooled human γ-globulin (1/50,000) and in highly purified pooled human IgG (1/15,000) (Table 2). These findings taken together strongly suggest that the factor is an antibody of the IgG class.

The fact that the augmenting antibody was found not only in heat-inactivated serum, but at serum dilutions far in excess of those detected by complement-dependent cytolytic antibody assay (not greater than 1/100 in our laboratory), indicated that the classical complement sequence is not necessary for expression of activity. The participation either of complement proteins produced by the MC in our assay system[15,16] or of an alternative pathway using complement components[17] cannot, however, be excluded. It is interesting that Wunderlich *et al*[18] found that addition of exogenous human complement does not enhance antibody-dependent cell-mediated cytotoxicity (ADC) in an allogeneic target cell system.

Because ADC has been shown in other systems to be mediated by effector cells obtained from either nonimmune or immune individuals[8], it was interesting to compare the degree of augmentation by MC from HSV-1 immune and nonimmune donors. In the presence of a 1/500 dilution of pooled γ-globulin, cells obtained from five seropositive human donors and three seronegative donors showed $12.4\pm4.6\%$ and $13.3\pm2.2\%$ augmentation, respectively. Thus, the presence or absence of neutralising antibodies in an individual does not appear to influence the ability of his or her mononuclear cells to demonstrate the augmentation phenomenon.

The effector cell in our heterogeneous Ficall-Hypaque-purified MC population is unknown. The similar cytotoxicity of nonimmune and immune mononuclear cells in our system as well as the augmentation rather than blocking of cytotoxicity by immune serum suggest that it is not the T lymphocyte. Furthermore, in a nonviral system, the effector cells for ADC in the rat are thymus-independent[19]. The monocyte[20], an adherent cell, is a possibility, however, in other models of ADC, the effector cell is a nonadherent cell[19,21], and preliminary experiments in our laboratory also indicate that the effector cell in our system is nonadherent to a plastic surface. The likely non-adherent effector cell, therefore, probably is a B lymphocyte or the recently described null lymphoid cell[22]. Attempts are now being made in our laboratory to identify the specific effector cell.

The cytotoxic effect of mononuclear cells and immune serum on HSV-1-infected cells is similar to the synergistic or antibody-dependent cytoxicity models described for unsensitised or antigen-coated chicken erythrocytes[8], Chang liver cells[9], allogeneic cells[18,23] and animal tumour cells[10]. These findings therefore extend the possible role of serum arming factors[10] to the immune damage of cells undergoing acute virus infection.

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Cyclic GMP stimulates lymphocyte nucleic acid synthesis

AGENTS that trigger proliferation of lymphocytes[1] and other cultured cells[2] have also been reported to stimulate increases in intracellular guanosine 3′,5′-monophosphate (cyclic GMP). These observations raise the interesting possibility that endogenous cyclic GMP acts as the intracellular mediator of some of the effects of mitogenic agents. If so, then it might be possible to demonstrate experimentally that cyclic GMP, introduced exogenously, has a mitogenic effect. We tested this possibility in mouse splenic lymphocytes and compared the mitogenic effects of cyclic GMP and two of its synthetic derivatives, 8-bromo-cyclic GMP (8-Br cyclic GMP) and N^2 O^2-dibutyryl-cyclic GMP (dibutyryl cyclic GMP), with that of concanavalin A (con A), a mitogenic lectin reported[1] to increase the cyclic GMP content of human lymphocytes. Our results indicate that, like con A, cyclic GMP and its derivatives can stimulate RNA and DNA synthesis in lymphocytes.

Splenic leukocytes from BALB/c mice were prepared as previously described[3] and cultured in serum-free RMPI-1640 medium, at 37° C in an atmosphere of 5% CO_2, 95% air. After addition of nucleosides or con A to the cultures, stimulation of

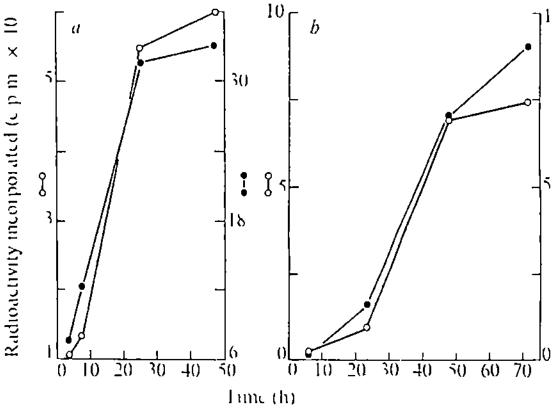

Fig 1 Time course of incorporation of (*a*) ^{3}H-uridine (26 Ci mmol^{-1}, New England Nuclear Corp.) and (*b*) ^{3}H-thymidine (15 Ci mmol^{-1}, New England Nuclear Corp.) into acid-precipitable macromolecules of spleen cells exposed to 8-Br cyclic AMP (Sigma), 1×10^{-3}M (○), or con A (Miles-Yeda), 0.5 μg ml^{-1} (●). Cells (2×10^6 per tube) were incubated in 0.5 ml RPMI-1640 medium (Grand Island Biological Co.). At the times indicated on the abscissa, 2 μCi of ^{3}H-labelled precursor was added to the culture and incubation was continued for an additional hour (for uridine) or 2 h (for thymidine), then 2.5 ml 5% TCA was added. The cells were centrifuged and washed two times with 5% TCA, then dissolved in 0.2 ml NaOH, and counted in liquid scintillation solution. Each point represents the mean of triplicate cultures, differing by not more than 10%, the quantity of radioactivity incorporated into cells in parallel cultures not treated with drugs has been subtracted (3,500 c.p.m. for uridine, 600 c.p.m. for thymidine).

Table 1 Effect of cyclic nucleotides and con A on thymidine incorporation

Additions	Control*	Dibutyryl cyclic AMP, 2 × 10^{-4}M
None	2 4	3 4
8-Br cyclic GMP, 1 × 10^{-3} M	13 2	12 3
Con A, 0 5 μg ml^{-1}	89 1	16 2
Con A + 8-Br cyclic GMP	107 3	34 0

Culture conditions and assay are described in the legend to Fig 1 Thymidine incorporation was measured in triplicate at 44 h Figures are c p m × 10^{-3}

*In these experiments dibutyryl cyclic AMP was omitted

nucleic acid synthesis was assessed at intervals by measuring the incorporation of pulses of radioactive uridine or thymidine into trichloroacetic acid (TCA)-precipitable material

8-Br cyclic GMP caused time-dependent increases in RNA and DNA synthesis, as indicated by uridine and thymidine incorporation, respectively (Fig 1) Stimulation by 8-Br cyclic GMP paralleled temporally that produced by con A, although the lectin produced quantitatively greater effects Uridine incorporation increased early with both 8-Br cyclic GMP and con A, and reached a plateau at about 25 h (Fig 1*a*) The increase in thymidine incorporation, which was initially detectable at 20 h, plateaued at about 46 h (Fig 1*b*)

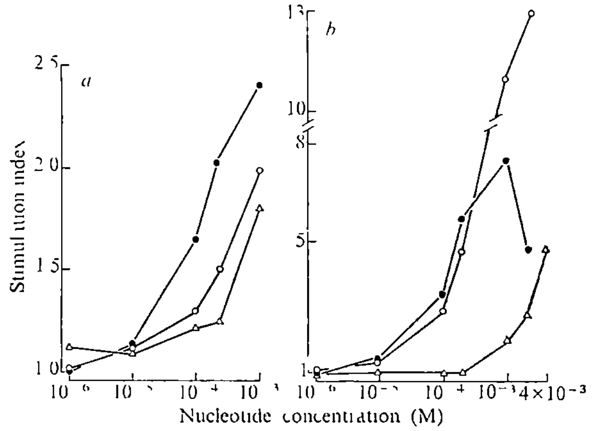

Fig 2 Stimulation of ^{3}H-uridine (*a*) and ^{3}H-thymidine (*b*) incorporation into TCA-precipitable macromolecules by various concentrations of guanosine 3′,5′-cyclic nucleotides △, cyclic GMP, ●, dibutyryl cyclic GMP, ○, 8-Br cyclic GMP Stimulation index (ordinate) is the ratio of incorporation by nucleotide-treated cultures to that by cultures without adding drug (4,100 c p m for uridine and 1,200 c p m for thymidine) Incorporation was measured at 20 h for uridine and 44 h for thymidine Triplicate points agreed within 10% The incorporation assay and culture conditions are described in the text and the legend to Fig 1 Viability of cells (assessed by exclusion of trypan blue) was not affected by the nucleotides at concentrations shown The spleen leukocytes in these experiments were 90–95% lymphocytes, contaminated with 5–10% macrophages and some erythrocytes Identical results were, however, obtained if erythrocytes were removed by osmotic lysis and macrophages removed by adsorption to Petri dishes or glass wool

The stimulatory effects of 8-Br cyclic GMP, dibutyryl cyclic GMP, and cyclic GMP itself, were dose dependent (Fig 2) At 1 × 10^{-3} M the cyclic GMP derivatives produced a 2 to 2 5-fold stimulation of uridine incorporation and an 8 to 11-fold increase in thymidine incorporation Cyclic GMP was less potent and produced mitogenic effects at high concentrations only (4 6-fold stimulation of thymidine incorporation at 4 × 10^{-3} M) 5′-Guanosine monophosphate did not stimulate thymidine incorporation at concentrations between 10^{-4} and 4 × 10^{-3} M (results not shown)

Since endogenously synthesised adenosine 3′,5′-monophosphate (cyclic AMP) and exogenous $N^6,O^{2'}$-dibutyryl cyclic AMP (dibutyryl cyclic AMP) have been reported to inhibit the lectin-induced mitogenic activation of lymphocytes[4,5], we asked whether dibutyryl cyclic AMP could inhibit the stimulatory effects of 8-Br cyclic GMP The dibutyryl cyclic AMP derivative was used at a concentration (2 × 10^{-4} M) that maximally inhibited con A-stimulated thymidine incorporation without reducing cell viability (measured by exclusion of trypan blue) Dibutyryl cyclic AMP did not affect the stimulation of thymidine incorporation caused by 1 × 10^{-3} M 8-Br cyclic GMP although it did inhibit stimulation by con A by 80% (Table 1) In addition 8-Br cyclic GMP did not protect con A-stimulated cells from inhibition by dibutyryl cyclic AMP (Table 1) In fact the stimulation of thymidine incorporation by 8-Br cyclic GMP appeared to be approximately additive to that caused by con A (at a concentration found in preliminary experiments to be maximally effective) whether or not dibutyryl cyclic AMP was present It appears likely that the cyclic GMP derivatives stimulate a subpopulation of splenic lymphocytes smaller than that stimulated by con A

It has been proposed that cyclic AMP and cyclic GMP act in opposite directions in biological systems, so that the ratio of the two nucleotides within cells regulates many cell functions, including growth[6] Cyclic AMP inhibits the growth of cells in culture[7], as well as the lectin-induced transformation of lymphocytes[4,5] We have found that exogenous cyclic GMP and two of its chemical derivatives can stimulate resting mouse spleen lymphocytes to synthesise RNA and DNA, with a time course similar to that caused by con A

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Do immunoglobulins have proteolytic activity?

As more and more proteins are sequenced, the search for sequential and structural interrelationships among them is becoming a major activity, even though it is still in its infancy compared with investigations dealing with ancestral relationships[1,2] For our study of homologous regions found among ancestrally unrelated proteins we have modified McLachlan's double matching method[3] and used it to find the statistically significant homologies The method, the details of which are being published elsewhere[4], enables a computer to perform a sliding match between two proteins, either when both proteins are aligned from N-terminal to C-terminal, which we call 'normal matching', or when one is aligned from N-terminal to C-terminal while the other is aligned from C-terminal to N-terminal, which we call 'reverse matching' At each step during this sliding match, some amino acids will be found to be identical between the two proteins being matched and some others will be different Based on similarities found among ancestrally related proteins the probability of each amino acid being substituted by another is calculated—the *M* score of similarity[3]—and printed The scores reach from 0, a non-match, all the way to 8 and 9 both of which stand for exact matches, 9 being assigned to phenylalanine, cysteine, tyrosine and tryptophan For ease of detection, the printout has a stop ( ) for a score of 8 and a prime (′) for a score of 9 (ref 4) The same molecule can also be matched against itself to reveal the presence of repeating subsequences and symmetry patterns[5]

During this study it was observed that the serine-containing active site fragment of bovine trypsin as well as of *S griseus* proteolytic enzyme matched the variable region of Bence Jones protein λ SH with an exceptionally high *M* score corresponding to a gaeat statistical significance (Tables 1 and 2) More than one homologous region was observed between the histidine, aspartic acid and serine-containing active site fragments of trypsin and Bence Jones λ human SH, in other words there were multiple copies of each trypsin active site fragment with varying degrees of significance, in Bence Jones λ human SH This suggests to us the possibility for the repeating sequences found in many proteins[6] to be related to a limited number of primordial subsequences from which all proteins could have evolved[5] We are not aware of any report which links any proteolytic activity with immunoglobulins The level of significance that was obtained between Bence Jones λ human SH and serine-containing active site fragments of various proteolytic enzymes, however, is so great that we decided to compare the distances between the serine, aspartic acid and histidine active site fragments of trypsin with the distances between various homologues of these fragments on Bence Jones λ human SH

Table 1 Significant homologies found between the Bence Jones λ human SH and serine-containing active site fragment of various proteolytic enzymes

Target protein	Position number	Matching sequences Individual scores	*M* score	Double matching probability
S grieseus enzyme	*	TCQGDS ′	49	8 8 × 10^{-9}
Trypsin, bovine	183	SCQGDS 5′	46	8 8 × 10^{-8}
Chymotrypsin A & B, bovine	195	SCMGDS 5′ 3 .	41	5 7 × 10^{-6}
Key protein				
Bence Jones λ SH	20	TCQGDS		

Target represents the protein(s) matched and key the protein which is used to find the matching sequences among the target proteins Position number indicates the position along the protein, of the sequence being matched, numbered contiguously from the amino terminal of the protein Contiguous numbering is used to avoid confusion arising from many different groups introducing gaps into their sequences for various reasons The numbers, periods and primes below the target sequences represent the individual similarity scores between the amino acids of the target and the amino acid of the key directly below it *M* score shows the cumulative score for the span length of the key, which is obtained by summing of the individual scores Double matching probability gives the probability for such a matching occurring by chance The f_A used in this matching represents the composition of amino acids of the key[3] and f_B the composition of the universe of proteins given in ref 1 according to ref 4

The single letter amino acid alphabet is used[1], for the sake of indisputable clarity, when alignments are given S, serine, C, cysteine, Q, glutamine, D, aspartic acid, T, threonine K, lysine, N, asparagine, A, alanine, F, phenylalanine, R, arginine, M, methionine

* Not known since only fragments have been sequenced

Table 2 Significant homologies found between serine-containing active site fragment of trypsin and different Bence Jones proteins

Target protein	Position number	Matching sequences Individual scores	*M* score	Double matching probability
Bence Jones λ human SH	20	TCQGDS 5′	46	8 8 × 10^{-8}
Bence Jones human HA and NEW	21	SCSGGS ′3 3	39	4 7 × 10^{-5}
Bence Jones human VIL and BO	21	SCTGTS ′3 3	39	4 7 × 10^{-5}
Bence Jones human X and BAU	20	TCSGDK 5′3 3	36	2 5 × 10^{-4}
Bence Jones human Kern	20	TCSGDN 5 ′3 5	38	6 0 × 10^{-5}
Bence Jones mouse 104E	21	TCRSST 5′5335	30	5 4 × 10^{-3}
Key protein				
Trypsin	183	SCQGDS		

Legend as Table 1

A close examination of the three dimensional model of an immunoglobulin made it possible to eliminate many of these possibilities leaving only two regions where the sequences found homologous to the three active site fragments of trypsinogen are reasonably close to each other One of these regions is located at the amino-terminal end of the light chain (the variable region) completely exposed to the outside The distances between the three amino acids serine aspartic acid and histidine are given in Table 3 The other area is in the constant region of the light chain but completely buried inside the molecule (Table 3)

The distances between trypsin active site fragments, calculated from atomic coordinates, is also given in Table 3 Whether any immunoglobulin molecule would exhibit proteolytic activity can only be determined experimentally We speculate that the area in the variable region of the immunoglobulin exposed to the outside might be the site for proteolytic activity, which would possess very little selectivity Perhaps a peptide would be needed to bind to this area and bring the distances between the indicated amino acids closer to the distances found between serine, histidine and aspartic acid fragments of trypsin

Table 3 Distances (in Å) between the active site residues of trypsin and homologous residues of Bence Jones λ human SH

Trypsin	$S_{188}-H_{46} = 8\ 7$	$S_{188}-D_{90} = 11\ 0$	$H_{46}-D_{90} = 6\ 1$
Bence Jones	$P_7-S_{71} = 16$	$P_7-S_{26} = 22$	$S_{26}-S_{71} = 7$
Bence Jones	$H_{176}-S_{198} = 14$	$H_{176}-D_{200} = 18$	$S_{198}-D_{200} = 4$

The distances for trypsin were calculated from its atomic coordinates The distances for Bence Jones λ human SH were measured from its model The first set represent the homologous sites at the variable region of the immunoglobulin molecule and the second set the constant regions (see text) Numbers indicate the position of the amino acid contiguously from the N-terminal of the protein

A recent study[7] has demonstrated that mutants of *Escherichia coli* K12 with deletions of *lac Z* gene can re-acquire the ability to hydrolyse β-galactosides Full lactose competence was restored through a sequence of at least five mutations The enzyme evolved differed from the *lac Z* β-galactosidase of the wild type *E coli* in its immunological, kinetic and sedimentation characteristics The authors suggested that the adaptation of a gene not involved in lactose utilisation to a form capable of specifying a β-galactosidase is similar to evolution by natural selection

We believe that the gene(s) coding for the protein whose active site fragment(s) has a three dimensional conformation closest to the active site fragment(s) of the wild type β-galactosidase is the one which underwent five or so mutations to yield the new enzyme[5]

We thank Dr R J Poljak for the use of his molecular model of the immunoglobulin molecule, Drs R M Stroud and R E Dickerson for sending the atomic coordinates of trypsin and Drs J P Glusker and H M Berman for providing help to obtain interatomic distances between active site fragments of trypsin

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Specificity of antigen receptors for cytochrome *c* in delayed hypersensitivity

The specificity of receptors on thymus-derived cells (T) and bone marrow-derived cells (B) for the same antigen is of interest if the various immune responses directed towards protein antigens[1] are to be understood Detailed molecular studies of such specificities can only be undertaken with protein antigens of known structure Following a large body of detailed work on the antigenic structure of cytochrome *c* for determinants related to antibodies[2], we report here the beginnings of a similar approach to an analysis of the determinants in cytochrome *c* related to delayed hypersensitivity

Female albino Hartley guinea pigs (400–450 g) were immunised with 1 0 mg of horse cytochrome *c* in 0 2 ml phosphate buffered saline (PBS) (emulsified with an equal volume of Freund's complete adjuvant (FCA)) by the injection of 0 1 ml into each footpad Two weeks later the animals were skin tested with 0 1 mg of horse cytochrome *c* in 0 1 ml PBS The sites of injections were assessed for thickness with a caliper (Schnelltaster, System Kroplin AO2T, Kroplin, Schluchtern, Germany) at 2, 4, and 24 h, as was the diameter of erythema No significant reactions were noted at 2 or 4 h and injection of unimmunised guinea pigs had no significant effects at any time interval In addition to the absence of 'immediate' type skin reactions, a search for specific antibody was made by several methods and in every case found to be negative, as has previously been reported[3] The sera for testing were taken 4 d following the skin test dose in 15 consecutive animals Passive haemagglutination using the glutaraldehyde method for coupling[4] the cytochrome *c* to sheep erythrocytes was used and titrations were carried out in microtitre plates All 15 sera had a zero titre while a positive control rabbit antihorse *c* (cytochrome *c*) serum containing 0 1 mg antibody protein ml^{-1} had a titre of 1 160 with such cells Passive cutaneous anaphylaxis (PCA) was carried out with three serum pools from groups of five animals 0 1 ml of such pooled sera at a 1/5 dilution gave negative PCA reactions Finally, all 15 sera were tested by a radioimmunoassay double antibody method[5] A potent rabbit anti-guinea pig γ globulin serum was used as second antibody In all 15 sera, 2 μl of immune guinea pig serum bound less than 5% of a tracer dose of 20 ng of ^{131}I-horse cytochrome *c* We thus concluded that in these animals at the time of testing no detectable circulating antibody was present

On the other hand, all animals had significant delayed reactivity at 24 h with erythema averaging 1 3 cm (± 0 5) in diameter and in duration averaging 0 65 mm (± 0 3) The specificity of this reaction was then studied by skin testing horse *c* immunised animals with various heterologous cytochromes *c* The results are illustrated in Fig 1 Skin thickness relative to the activity of horse *c* is plotted as a function of the number of amino acid differences existing between horse *c* and the heterologous *c* used for skin testing The specificity of the reaction is quite restricted Kangaroo, turkey, human, and tuna cytochromes *c* which differ from horse *c* by 7, 11, 12 and 17 amino acids, respectively, give no detectable skin responses in animals immunised with horse *c* Donkey, bovine, and sheep (the latter two cytochromes *c* have an identical primary sequence) cytochromes which differ from the horse protein by one, three and three amino acids, respectively, are indistinguishable from horse *c* in skin tests while whale *c* (which differs by five residues from horse *c*) is about one half as effective in the skin tests Dog and rabbit cytochrome *c* which differ by six residues from the horse protein are even less active than the whale protein As bovine cytochrome *c* exhibits all the skin reactivity of the horse protein, the three sequence differences between them are not the residues conferring specificity in this reaction The relevant sequence comparisons are listed in Table 1

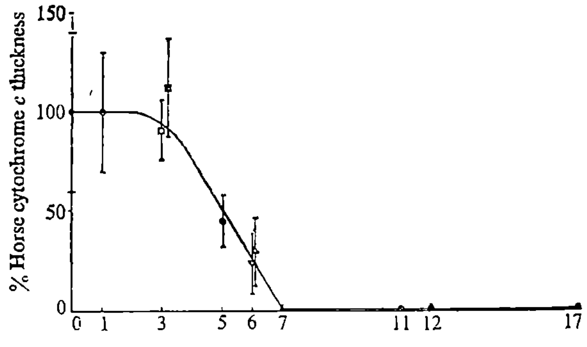

Fig. 1 Cross reactions of various heterologous cytochromes *c* in horse *c* immunised animals Horse (●), donkey (half filled circle,) bovine (half filled square), sheep (□), whale (diagonal crossed circle), dog (▽), rabbit (△), kangaroo (▲), turkey (diagonal crossed square), human (vertical crossed circle), and tuna (■) The vertical line through the symbol represents one standard deviation of the mean of the skin thicknesses, 24 h after skin test Sequence data from Dayhoff[16]

The two sequence differences between whale and bovine *c* are important, however, as are the two differences between the whale and rabbit proteins Sequence position 92 is of special interest since bovine and horse *c* both carry glutamic acid there, while the whale and rabbit proteins carry alanine In the same region at position 89, rabbit *c* carries aspartic acid while the bovine and whale proteins carry glycine Thus, there is an order of sequence similarity and differences which parallels the quantitative changes in specificity In the region encompassing sequence positions 89 and 92, bovine *c* is most like the horse protein, rabbit *c* is the most distantly related and whale *c* is intermediate in structure There are also amino acid differences at positions 44 and 62 which could contribute to the specificity differences exhibited by the bovine and whale proteins and between the whale and rabbit proteins, respectively

Table 1 Cytochrome *c* sequence comparisons

	44	62	89	92
Horse	Pro	Glu	Thr	Glu
Bovine	Pro	Glu	Gly	Glu
Whale	Val	Glu	Gly	Ala
Rabbit	Val	Asp	Asp	Ala

Sequence data from Dayhoff[16]

This structural specificity is related to these sequence differences in the context of the three-dimensional structure of cytochrome *c* The evidence for this is that the cyanogen bromide fragments of cytochrome *c* which comprise the sequences 1–65, 1–80, 66–80, and 81–104 are all inactive in eliciting delayed skin reactions in animals immunised with horse cytochrome *c* Such data suggest that the determinants responsible for the skin reactivity are very conformation dependent, a situation which is extremely common with antigenic determinants in globular proteins[6,7] The cross reactions in this delayed-hypersensitivity system are quite limited and, in fact, exhibit a narrower specificity than do the comparable cross reactions carried out with rabbit antibody to horse cytochrome *c* (ref 2)

This high specificity for the immunogen in cross reactions of delayed hypersensitivity is not restricted to the horse protein Guinea pigs immunised with either human or turkey cytochromes *c* do not cross react with horse *c* in skin tests The strength of the homologous reactions is comparable to that of horse *c*-immunised animals and no circulating antibody was demonstrable in either human or turkey *c*-immunised guinea pigs

Such high specificity has often been demonstrated in delayed hypersensitivity reactions involving the dinitrophenyl-oligolysine system[8] and a number of other systems involving haptenated autologous proteins[9,10] On the other hand, cross reactions of delayed hypersensitivity to proteins have generally shown a very broad specificity and frequently exhibit good to excellent cross reactions between antigens that do not measurably cross react with specific antibody[11–15] Thus, the cytochrome system exhibits the type of exquisite specificity more characteristic of delayed reactivity to haptenes than of the type generally characteristic of protein antigens

The cytochrome system would seem to be an ideal one for the study of the molecular relationship of those structures responsible for interaction with receptors of the two kinds of lymphocytes involved in immune responses

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Hepatitis B and serum DNA polymerase activities in chimpanzees

THE association of hepatitis B antigen (Australia antigen, HBs Ag) in human serum with DNA polymerase activity was first reported by Hirschman *et al*[1] This has been confirmed and extended by Kaplan *et al*[2] who found DNA polymerase activity in preparations of concentrated human hepatitis B antigen which were rich in Dane particles The DNA polymerase activity previously described by Hirschman *et al*, however, was considered to be dependent on an RNA template, whereas the DNA polymerase reported by Kaplan *et al* appeared to be a DNA-dependent DNA polymerase

No studies have been reported on serum levels of DNA polymerase activity in subjects experimentally infected with aetiological agents of hepatitis B We have infected chimpanzees with hepatitis B viruses[3] to study the temporal relationship between DNA polymerase activity in serum and the appearance of HBs Ag Four susceptible chimpanzees were inoculated intravenously with sera containing hepatitis B antigen Each chimpanzee was monitored for serum HBs Ag and corresponding antibody (anti-HBs) during the course of infection by a solid-phase direct radioimmunoassay (RIA) system[4] Liver damage was assessed by measuring serum glutamic pyruvate transaminase (SGPT) activity and by histological examination of liver biopsy tissue Serum samples were obtained under anaesthesia three times weekly, and assayed for DNA polymerase and HBs Ag or anti-HBs For DNA polymerase we used a modification of the method of Robinson and Robinson[5]

Of four animals inoculated, three developed infections characterised by the occurrence of transient HBs Ag followed by anti-HBs production In all three hepatitis B was confirmed enzymatically and histologically The fourth animal did not develop HBs Ag or evidence of hepatitis but produced late-occurring anti-HBs 70 d after inoculation

In all four animals we found spikes of DNA polymerase activity, the first increases being uniformly observed between the 20th and 40th days after inoculation In the three animals who experienced antigenaemia and hepatitis, first spikes in DNA polymerase either closely preceded or paralleled the first appearance of HBs Ag and always occurred before first increases in transaminase levels Figure 1 shows these relationships for one of the three animals (Ike), and reveals a second DNA polymerase peak post-dating the bulk of the antigenaemia and closely preceding the transaminase peak In the other two animals experiencing hepatitis, increases in DNA polymerase activity clearly preceded the first appearance of HBs Ag (by 20 d in one animal, and by 25 d in the other) and similar secondary

Table 1 Effect of reaction conditions on DNA polymerase activity (Ike) in two serum pools

	Complete, no additions	−NTPs	+Act D	+DNase	+RNase	+poly (dA-dT) poly (dA-dT)
Day 26 Pool	129*	36	23	79	121	77
Day 33 Pool	411	137	50	128	405	304

*Net c p m (gross c p m less background and zero-time c p m)

Pools of serum obtained on days corresponding to DNA polymerase peaks occurring on days 26 and 33 were tested for the effects of various additions and deletions on the incorporation of ^{3}H-dTTP into a TCA-insoluble product Final reaction-mixture concentrations of the additions were actinomycin D, 10 µg ml^{-1}, DNase (EC 3 1 4 5), 10 µg ml^{-1}, 30 min at 37° C, RNase (EC 2 7 7 16), 10 µg ml^{-1}, 30 min at 37° C, poly(dA-dT) poly(dA-dT), 25 µg ml^{-1}

polymerase peaks appeared in both and coincided with increases in transaminase levels

As Fig 2 shows, the serum of the fourth animal (Jay) showed spikes in DNA polymerase activity between the 20th and 40th days and the 50th and 70th days after inoculation in the absence of detectable HB$_s$ Ag or increases in transaminase activity Anti-HB$_s$ was first detectable on day 70

Characterisation of the two DNA polymerase activities found in Ike's serum is summarised in Table 1 Both were inhibited by actinomycin D (10 µg ml^{-1}) or poly(dA–dT) poly(dA–dT) (25 µg ml^{-1}), indicating that these activities do not depend on an RNA template Preincubation of the day 26 pool and the day 33 pool reaction mixtures (Table 1) with ribonuclease did not affect the incorporation of ^{3}H-TMP into trichloroacetic acid (TCA)-precipitable products, further suggesting that these polymerase activities do not depend on an endogenous RNA template The products of the polymerase reactions, however, were partially sensitive to DNase Omission of nucleoside triphosphates from either of the reaction mixtures resulted in decreased incorporation of ^{3}H-dTTP into acid insoluble products

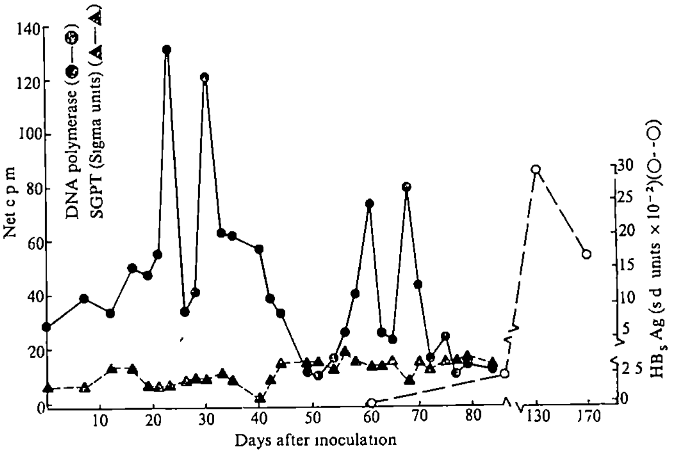

Fig 2 Chimpanzee Jay Serum assays are identical to those in Fig 1, except that 15 mM Mg^{2+} was substituted for 5 mM Mg^{+2}, the final assay was 0 1% in Triton X-100

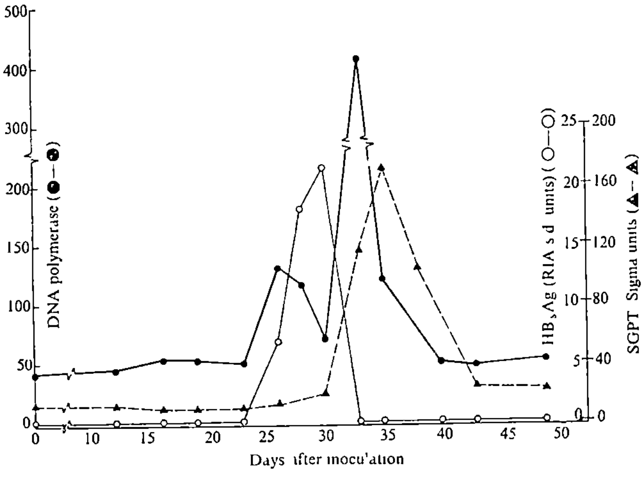

Fig 1 Chimpanzee Ike DNA polymerase activity in serum was determined by a radioisotopic method which measures the incorporation of ^{3}H-dTTP into TCA-insoluble product The polymerase assay contained in a volume of 0 300 ml 50 µl serum, 200 µl 0 1 M Tris-HCl buffer, pH 8 3, the balance of the reaction mixture contained (final assay concentrations) mercaptoethanol (2 5 mM), dATP, dCTP, dGTP (0 25 mM each), MgCl$_2$ 6H$_2$0 (5 mM, unless otherwise noted), KCl (40 mM), and ^{3}H dTTP (0 93 µM) The reaction mixture was incubated for 60 min at 37° C, 100 µl of the mixture was pipetted into 5 ml of ice-cold 5% TCA+2% pyrophosphate solution to precipitate the labelled product The precipitate was collected on a Millipore filter (EAWPO2500) and washed repeatedly with 5% TCA+2% pyrophosphate, 80% ethanol and dried with diethyl ether The disk was placed in 10 ml of toluene PPO-POPOP cocktail for counting Background and zero-time assays c p m were subtracted to yield net c p m Serum glutamic pyruvate transaminase (EC 2 6 1 2) was measured by colorimetric Sigma procedure Serum HB$_s$ Ag was determined by a solid-phase direct radioimmunoassay (AusRIA, Abbott Laboratories) Results for HB$_s$ Ag are expressed in standard deviation (s d) units Preinoculation chimpanzee serum polymerase activity averaged 24 c p m

Based on the results of our study of DNA polymerase activity in four chimpanzees infected with hepatitis B viruses, we believe two preliminary conclusions can be drawn First, for animals experiencing hepatitis, DNA polymerase activity appears in the serum before the onset of antigenaemia and liver damage and may represent replication of viral DNA Second, the postulated viral DNA polymerase activity (in Ike) seems to depend on a DNA template rather than on an RNA template—this idea is consistent with the findings of Kaplan *et al*, who demonstrated that DNA polymerase activity associated with the Dane particle was inhibited by actinomycin D and daunomycin Furthermore, the detection of increased DNA polymerase activity in an infected animal (Jay) who showed no clinical, enzymatic (SGPT) or immunological (HB$_s$ Ag) evidence of hepatitis during the first 70 d after inoculation suggests the DNA polymerase assay may be useful in screening potentially infective sera

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Release of enzymes from cell walls by an endopectate-*trans*-eliminase

THE cell walls of higher plants were long regarded as essentially inert structures which functioned mainly as mechanical barriers to the expansion of protoplasts under turgor pressure and as skeletal supports in tissues It is now known, however, that they contain a number of hydrolytic enzymes at least one of which is a hydroxyproline-rich protein[1] These enzymes are often of types associated with the dynamic recycling functions of intracellular lysosomes[2-4] They have usually been detected by the activity of cell wall preparations after comminution of tissues and removal of contaminating cytoplasm by washing[1,2,5] or by the activity of intact or excised roots[3,6] We now report on the release of wall bound enzymes from living cells by a pectic enzyme

An endopectate-*trans*-eliminase (PTE) was obtained from 3-d-old soft rots of cucumbers caused by the bacterium *Erwinia carotovora* (NCTC 1747) One volume of rot was extracted with one volume of 0 85% NaCl and made cell-free by centrifuging Ammonium sulphate was added to 61–80% saturation, the precipitate collected, dissolved in, then dialysed against water, and fractionated by elution from Sephadex G75 superfine with 0 1 M NaCl in 0 005 M Tris buffer, *p*H 9 0 Fractions containing most of the PTE activity were subjected to isoelectric focusing between *p*H 7 0 and 10 0 for 52 h at 4° C Fractions containing PTE were combined, dialysed and gave a single band after electrophoresis on polyacrylamide gel The preparation did not degrade any of a wide range of cell wall and other polymers other then those based largely on anhydrogalacturonic acid residues One unit of activity of this PTE gave an increase in absorbance at 550 nm of 0 1 in 30 min at 25° C in the following assay 1 ml enzyme solution, 2 5 ml 1% sodium polypectate (Sunkist Growers Inc), 0 5 ml $CaCl_2$ (final 0 001 M), Tris buffer, *p*H 9 0 (final 0 1 M), left at 25° C Then 7 5 ml of a mixture of 5 ml thiobarbituric acid (0 04M) and 2 5 ml HCl (1M) was added and the red colour produced estimated at 550 nm against a reagent blank

Table 1 Effect of endopectate-*trans*-eliminase (PTE) on release of K^+ and enzymes from potato tuber tissue

Activity†	Treatment*					
	a	*b*	*c*	*d*	*e*	*d*+*e*
K^+	625	17	685	715	60	775
Acid phosphatase	0 132	0 059	0 056	0 040	0 121	0 161
Phenoloxidase	0 13	0 032	0 059	0 046	0 12	0 166
Peroxidase	0 10	0 060	0 023	0 008	0 083	0 091
Malic dehydrogenase	0 132	0 082	0 115	0 052	0 22	0 272

Values shown are corrected for slight changes caused by autoclaved enzyme

* *a*, 3 ml water containing 14 8 units PTE, 0 001 M $CaCl_2$, 0 02 M Tris buffer (*p*H 9 0) for 3 h at 22° C, *b*, as *a* but in 0 5 M sucrose as plasmolyticum, *c*, as *b*, then liquid removed, disks washed in 0 5 M sucrose, added 3 0 ml water for 10 min, *d*, 3 ml, frozen at −20° C, thawed and kept for 10 min, *e*, liquid removed, disks then treated as *a*

† K^+ activity by flame photometry (p p m) Acid phosphatase by estimating *p*-nitrophenol liberated from *p*-nitrophenyl phosphate at 400 nm (ref 7), activity was expressed as mg *p*-nitrophenol liberated per 0 2 ml enzyme solution Phenoloxidase by estimating oxidation of catechol[8], activity was expressed as Δ *OD* 495 nm min^{-1} per 0 2 ml enzyme solution Peroxidase by estimating oxidation of pyrogallol in the presence of H_2O_2 (ref 9), activity was expressed as Δ *OD* 420 nm min^{-1} per 0 01 ml enzyme solution Malic dehydrogenase by estimating oxidation of NADH in the presence of oxalacetic acid[10], activity was expressed as Δ *OD* 340 nm min^{-1} per 0 1 ml enzyme solution

Samples of 48 disks (0 5 × 7 0 mm diameter) from medulla of turgid potato tubers (cv Majestic) were treated in various ways

At the end of each treatment, the ambient liquid was collected and assayed for K^+, acid phosphatase, phenoloxidase, peroxidase and malic dehydrogenase

The 3 h treatment of tissues with PTE caused cell separation and death of protoplasts in the sense that they did not plasmolyse in hypertonic solutions, treatment with enzyme in a plasmolyticum still caused cell separation but protoplasts remained plasmolysed and intact

Table 1 summarises the effects of the different treatments and also shows in column *d* + *e* the total of K^+ and enzymes liberated from frozen tissue after thawing and released from residual tissue after treatment with PTE for 3 h Comparison of columns *d* and *e* show that K^+ liberated by PTE treatment of residual tissue was only about 10% of that liberated by freezing followed by thawing The corresponding figures for the enzymes were 72–91%

Similarly, although very little K^+ was released from tissues treated with PTE in a plasmolyticum, large amounts of peroxidase acid phosphatase and malic dehydrogenase and smaller though substantial amounts of phenoloxidase were liberated in these conditions There were corresponding differences when plasmolysed protoplasts in PTE treated tissue were rapidly deplasmolysed

Because PTE is believed to act only on substrates in cell walls and middle lamellae, the results suggest that large proportions of the four enzymes in cells are bound to cell walls and are liberated when these are exposed to PTE This confirms earlier work which showed that cell walls contain substantial amounts of peroxidase[1] and acid phosphatase[5] but is unexpected for malic dehydrogenase (which is known to occur as different isoenzymes in mitochondria, chloroplasts and cytoplasm[11] but not, so far as we know, outside the plasmalemma)

We have considered the possibility that our results could be explained, at least in part, by adsorption by cell walls of cytoplasmic enzymes released when cells are broken during preparation of the potato tuber disks or by residual cytoplasm on cut surfaces But this seems unlikely because preliminary washing of disks in 0 2 M NaCl did not decrease release of acid phosphatase or malic dehydrogenase when disks were later treated with PTE in a plasmolyticum It seems, therefore, that these four enzymes are released from cell walls by the action of PTE which is highly specific and is believed to be confined to the rupture of α-1,4-D-glycosiduronic linkages This poses the question of the relationship between polymers based on these linkages and wall-bound protein and, of course, the function of wall-bound enzymes particularly such as malic dehydrogenase hitherto regarded as essentially cytoplasmic

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reviews

Simple guide to welfare economics

In Defence of Economic Growth By Wilfred Beckerman Pp 287 (Cape London, June 1974) £3 95

THIS book is precisely what it claims to be a defence of economic growth It is written with wit and verve, in a forensic style which would do credit to a barrister defending a criminal before a jury The prosecution witness (the mass media, fuddy-duddy scientists, young radicals, and the middle classes) are battered by facts, arguments and ridicule It is, as Beckerman frankly warns his readers, a one sided contribution to a debate The issue is familiar enough Should economic growth be curtailed or even phased out as a deliberate social policy, in order to prevent pollution and to conserve resources for future generations? And, to anyone interested in this issue, Beckerman's views are familiar enough too Market forces, either working naturally or modulated by a system of taxation, will continue, as they do at present, to regulate the level of pollution and the use of resources Zero growth would exacerbate the inequalities between rich and poor, it would be of dubious benefit to future generations, and in any case it would be politically impossible to achieve in a democratic society

Many people, including some scientists, do not realise that the economist, dealing with the theory of choice in the allocation of resources, is (or tries to be) as neutral over value judgements as the chemist is when he deals with the theory of valency It is not the economist's professional business to moralise about how the national product should be allocated among such items as present consumption, investment in the future, and conservation of the environment His business is to say what the economic consequences will be for different patterns of allocation Thus in the mind of the economist pollution is not 'good' or 'bad' any more than arsenic is 'good' or 'bad' in the mind of a chemist The notion that conservationists have to defend the environment against hordes of economists, hell-bent on maximising economic growth, is, as Beckerman convincingly argues, nonsense The economist is interested in the logic, not the ethics, of choice

A clear distinction has to be made between two dimensions of choice one is how to allocate resources at any moment of time (for example, as between public and private transport), the other is how to allocate resources over a period of time (for example, as between 1975 and 1995) The controversy over economic growth lies in the second of these two dimensions It is, crudely, the choice between on the one hand, more consumption now and possibly (though Beckerman disputes this) less for our grandchildren and, on the other hand, some sacrifice of present consumption and (again only possibly) more for our grandchildren

The evidence of history is that improvement in economic welfare has been accompanied by improvement in social welfare As nations have become more affluent their citizens have, on the whole, become more healthy, enjoyed greater security, shared more fully those immaterial benefits we associate with 'quality of life' So, argues, Beckerman continued economic growth is in the best interests both of today's poor and of posterity As for the social costs of economic growth, for example pollution and disfigurement of the landscape, the economists have an elegant formula as a guide to policy Pollution (which Beckerman selects to exemplify his views) should not be minimised it should be optimised at the level above which the marginal cost of more abatement would exceed the marginal cost of the damage done if it were not abated And to ensure that the cost of disposing wastes into the environment is internalised, just as the cost of labour is, Beckerman proposes a hit-or-miss taxation system which would aim at optimising pollution

The trouble about the economists' elegant formula is that they cannot quantify it in a way which would command the confidence of anyone accustomed to the rigours of scientific method The costs of abating pollution can be measured But estimates of the damage done by pollution which try to quantify the disamenity of fogs and dirty rivers and smells are (to my mind) totally unconvincing Beckerman himself emphasises that economic welfare is only part of social welfare The task of politicians is to promote the whole of social welfare The issue which his book raises is a fundamental one Is social welfare so conditioned by economic welfare that politicians can exclude the unquantifiable components of social welfare? Or—and this is Beckerman's view—should one go on trying to quantify the whole of social welfare, in money terms because money is a convenient symbol of value? After all, he says, it was a long time before physicists learnt how to measure heat Or are the unquantifiable components in the cost-benefit equations of social welfare so important that this whole quasi-scientific approach to social policy ought, for the time being, to be regarded as under suspicion?

Beckerman, quite rightly, withers with criticism the specious precision of computer simulations about the prospects for mankind But is he not himself exposed to a somewhat similar, though much milder, criticism? There is growing evidence that social welfare includes much more than economic welfare and that in affluent societies the correlation between the two (let alone causation) cannot be assumed to be strong Much of what social welfare means cannot be quantified Is there not a risk that the value-free approach to welfare economics will over estimate the quantifiable components and under estimate the others and, in doing so, distort the issues instead of clarifying them?

One puts this challenging book down with the impression that economists, just because they are liable to become involved in social policy, cannot remain as disengaged from value judgements as scientists can The scientist can exclude from consideration phenomena he cannot quantify He would discard an equation for which he could not supply data The economist analysing social choice is tempted to go on using the equation even though he cannot supply the data Beckerman has not altogether resisted this temptation

E ASHBY

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Longman 1724-1974

Silent synapses

Memory and Nerve Cell Connections By Richard Mark Pp x+15 (Criticisms and Contributions from Developmental Neurophysiology) (Clarendon Oxford, Oxford University London, June 1974) £2 95

IN this very interesting book Richard Mark (well known for his work on selective neuromuscular reinnervation in fish and newts, in which he has shown that, in certain circumstances, neuromuscular synapses in lower vertebrates may become functionally switched off without apparent morphological change) extends his arguments on "silent synapses" to the central nervous system and attempts to provide thereby a skeletal framework for a theory of memory and learning The book is well organised, well written and should be read

The nature of the audience at which the book is aimed is not clear, since though the introductory outlines of the structure and function of neurones, glia, synapses, brain codes and so forth are somewhat elementary the arguments introduced later are fairly sophisticated This does not really matter, however, and students and research workers alike will find this book, which is organised rather like a detective story, highly readable After a brief survey of some relevant aspects of neurophysiology the author takes the reader on a tour of various possible mechanisms of memory (with their attendant disadvantages) and finally, eight pages before the end of the book, Dr Mark's own hypothesis, the component parts of which had previously been introduced, is put together The hypothesis essentially suggests that, in the highly convergent (and initially redundant) neural network of the central nervous system, the less used pathways may become functionally suppressed Used pathways may become more efficient and this increased usage is held to be associated with increased synthesis of specific marker proteins, leading to long term changes in functional connectivity

The general argument presented by Dr Mark is interesting and plausible He makes the good point that "it is not reasonable to abstract the verbal or logical representation of each component of a particular behavioural situation that can be used to demonstrate learning and then demand a physiological explanation for all these abstractions at the same biological level Yet many physiologically inclined workers have simply taken each of the parameters of a conventionally accepted learning paradigm such as classical conditioning and assumed that there is a cellular explanation for each of them" The point being made here is that the phenomena of learning and memory are highly complex and that simplistic attempts to identify individual structures with these functions are unlikely to be fruitful From a somewhat similar viewpoint Dr Mark briefly reviews the popular work on memory transfer ('memory molecules') and comes to the tentative conclusion that, although some of the experimental observations may be valid, the hypothetical frameworks associated with this sort of work have so far been inadequate "It would be imprudent to deny that chemically transmissable behaviour exists One has the uneasy feeling from all this literature, contradictory and incomplete as it is, that something is there"

Dr Mark makes the interesting criticism that, since the result of memory transfer experiments, even if valid, cannot be fitted into the present framework of biological understanding, the lack of coherence of these experiments with the other brain studies may rule out scientific progress along these lines "The most telling criticism of chemical memory transfer experiments is not to deny the possibility of the effects, or to denigrate the investigators, but to say that they are before their time This is just as serious a scientific criticism as to say the work is old-fashioned and repetitive It means that even if research stumbled upon something of real significance, which perhaps it already has the finding could not be exploited because of the surrounding chasms of ignorance as to other aspects of brain function"

Taking his example from the study of nerve regeneration in amphibians and fishes, Mark instances work on plasticity of synaptic connections which seems to be due to a competitive suppression of transmission in some terminals In these cases the final pattern emerges not because the best fitted connections improve but because the inappropriate are eliminated It is proposed that a mechanism akin to this developmental shaping of the nervous system may continue into adult life and work there to modify brain connections into a more appropriate form for current experience, that is, as a basis for learning

The details provided by Dr Mark to support this idea are, naturally somewhat scanty, and it may well turn out that the whole approach is misleading It is certainly true, however, that the existence of "silent synapses" peripherally, taken along with the recent evidence for functionally induced plasticity of central connections in the visual system, provokes very interesting speculations This may not be the most direct route to the mysteries of memory and learning, but the search for silent synapses in the CNS is now on •

R M GAZE

Materials in use

Metals, Ceramics and Polymers An Introduction to the Structure and Properties of Engineering Materials By Oliver H Wyatt and David Dew-Hughes Pp xiv+640 (Cambridge University London, May 1974) £12 00 boards, £4 95 paper

Of books on Shakespeare's plays, the Malaise of Western Man, and materials science, there is no end No blinding new insights are to be expected on any of these topics selection, clarity and economy must be our touchstones Anyone with a shelf-full of such books must be tempted to pluck out some of them and follow Prospero's heroic resolution "And deeper than did ever plummet sound I'll drown my book"

The volume under review is not for drowning It is one of the very best of the many English-language texts on materials that have appeared in recent years Unlike some of these, the new book has a pronounced engineering bias the fact that Dr Wyatt worked for years in an engineering school which prepares its students for what used to be called a Mechanical Sciences Tripos shows in the special emphasis on mechanical behaviour which is evident in many parts of the book Thus, although there is a sound summary of the present understanding of dislocation behaviour, macroscopic plasticity theory gets exceptionally thorough treatment, the application of this theory to metalworking processes is unusual in a materials textbook Ceramics and polymers each receive a good concise presentation, including for the former an informative account of the setting of concrete glasses are rather too much compressed (a state possibly natural to the material) There is an excellent chapter on steels, particularly thorough on the problem of heat-treating objects of various sizes The electrical and magnetic chapters are also well presented, with more emphasis than normal on superconductivity (one of Dr Dew-Hughes's special interests) and dielectric materials Magnetic materials place much more emphasis on domain behaviour than intrinsic magnetism—a good test of fitness for engineering purposes

The treatment of particular materials is underpinned by a fairly conventional introduction to topics such as crystallography, diffusion, phase diagrams (including a good treatment of ternaries) and band theory As so often, the thermodynamic treatment is too concise for real clarity, in particular, the statistical basis for entropy has to be taken on trust, which must tinge the crucial concept of an equilibrium population of vacant lattice sites with a touch of black magic But on the whole, the theoretical underpinning is very clear and sufficiently comprehensive

It is easy enough to pick out individual small inadequacies and as always these largely reflect the reviewer's predilections there is almost nothing on the important subject of recrystallisation, the intriguing explanation of how doping with an impurity permits the removal of pores in sintered alumina is muffed, the crucial importance of crystalline states in polymers receives only the most glancing recognition But the list of these shortcomings (which another reviewer might deem of little consequence) is short and the positive achievement of balance and clarity is very considerable

Each chapter has a collection of questions, partly numerical and partly purely verbal, designed to test conceptual understanding, and each has an excellent bibliography

As a basis for a comprehensive course on materials for engineering students, or as a foundation for the much smaller tribe of material specialists, this book can be warmly recommended The paperback version in particular is excellent value

R W Cahn

Valence calculations and molecular orbital theories

Ab Initio Valence Calculations in Chemistry By D B Cook Pp ix+361 (Butterworth London, March 1974) £7 50

This is a book which describes the technical aspects of computing wave functions and energies of electrons in molecules as approximate solutions of the Schrodinger equation It is concerned only with the *ab-initio* method, that is, the approximations contained in such calculations arise from the termination of series expansions of the wave functions rather than the use of a model Hamiltonian In addition to the technical aspects of the book the author gives a personal justification for the place of such calculations in chemistry which may be summarised as follows Many of the important concepts of valence theory are not observables, but in order to maintain contact with and influence the main stream of chemical thinking, quantum chemistry must apply itself to the analysis, criticism and quantitative investigation of these concepts In other words the book is not written just for chemists who want numbers, but also for those interested in ideas

The book fills a gap in the current literature It does not set out to present a broad view of quantum theory or valence theory and therefore assumes a fair amount of basic knowledge in these areas It is elegantly written and printed and I believe it will be widely accepted as a very useful book

Molecular Orbital Methods in Organic Chemistry—HMO and PMO An Introduction By William B Smith Pp xi+161 (Studies in Organic Chemistry, vol 2) (Dekker New York, April 1974) $14 50 £6 00

It is thirteen years since the first books on molecular orbital theories in organic chemistry appeared and one must admire the courage of both author and publisher in adding another volume to this crowded market About half the book is devoted to an elementary account of Huckel theory (HMO) and its perturbative approximations (PMO) The remainder gives a wide coverage of organic reactivity including the recently popularised symmetry rules As an elementary account of the subject it is quite acceptable but it is hardly value for money when compared with other books that are available

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Impact of climate

Climatic Resources and Economic Activity A Symposium Edited by James A Taylor Pp 264 (David and Charles Newton Abbot and London, June 1974) £6 50

THIS volume is the latest in a series emanating from the Aberystwyth Symposia, hardy annual gatherings from 1958 to 1973 These have focused attention usefully on what is broadly termed "applied climatology" Inevitably this volume overlaps the contents of certain of its predecessors in such an amorphous field, notably *Weather and Agriculture* (Oxford, 1967), *Weather Economics* (Oxford, 1970), and *Weather Forecasting for Agriculture and Industry* (David and Charles, 1972)

It claimed in the preface that this book is timely in reviewing relationships between climatic resources and some of their social and economic usages this is indisputable, although the range of papers it contains is broad rather than all-embracing, and the depth is distinctly moderate Many workers may take greater exception to the unqualified assertion that the volume is "representative of current research trends and achievements" in environmental science There are other kinds of trends and achievements too, several arguably of greater significance in the long run Perhaps the most unnecessary aspect of the introductory keynote is its attempt to justify this collection of articles in terms of current geographical philosophy One wonders whether the three-quarters of the contributors who are employed outside academic geography welcome being seen in this perspective If applied climatology and meteorology are fields worthy of investigation, they may be explored best if the directions from which they are approached are totally unprejudiced

The contents include a broad introduction to the concept of the atmosphere as a global resource, and five chapters of bioclimatic interest, almost wholly concerned with weather, plants, and crop husbandry Two further chapters—more helpful and interesting than most—appraise water resources in the United Kingdom, and mathematical models designed to facilitate the planning of their exploitation Two chapters follow on air pollution, then a rare, thought-provoking essay on weather and road accidents The two last contributions are concerned with modelling influences of atmospheric behaviour on general and national economical activities

Several chapters suffer from the current abuse of statistical methods for environmental analysis Correlations are based on tiny populations, and rakish linear regression lines are employed where curvilinear relationships even of only quadratic or cubic forms would have yielded much more significant values of r^2 Why weaken a potentially important argument if the chosen "best fit" statistical statement does it less—even much less—than justice?

As an ardent disciple of the school which prefers its scientific books to be more akin to disposable paper cups than presentation clocks, I am bound to think that the circulation of this book will be restricted by its style and cost It is too lightweight and piecemeal to serve as a worthy reference volume on the library shelves, meanwhile, it is too wasteful in production and design to recommend itself to the college student or the weather-conscious layman Presumably, therefore, it is aimed at the business market, where its value will depend on the nature of the enterprise A paperback version could have been recommended to students

Both front and back cover pictures are from the 1960s, which perhaps might just be forgiven But the satellite photograph of South America on the back cover would look considerably better—and make more sense to the amateur—if it were not upside down

E C BARRETT

Climate and Life By M I Budyko English edition edited by D H Miller Pp xvii+507 (International Geophysics Series, vol 18) (Academic New York and London, April 1974) $35, £16 45

THIS book provides the most complete account in English of the climatology work at the Voeikov Geophysical Observatory, and with very complete references at the end of each chapter it is important simply as a reference guide to this work in the Soviet Union

Apart from this, it is a book which will be of real value to the serious student of climatology, although it is certainly not always easy to read Starting from solar radiation and heat balance at the Earth, the study of climate and its impact on life is considered within a unified framework which builds upon the work reported in the earlier (1956) summary of research at Voeikov Observatory

I found the sections on climatic change particularly interesting and useful, I would have preferred to find less emphasis on the effects of volcanic dust, perhaps—or at least comparable emphasis on other factors—and I would take issue with the statement that "over a duration of several centuries, noticeable fluctuations of climate can occur only in high latitudes" But these are relatively minor points and in a book covering so much it is no surprise if the author fails to please all of the readers all of the time

JOHN GRIBBIN

Earth science

Geology, Resources and Society An Introduction to Earth Science By H W Menard Pp xi+621 (A Series of Books in Geology) (Freeman San Francisco and Reading, May 1974) £6 10

FROM the days of Lyell to those of Arthur Holmes and Jim Gilluly students of geology have been fortunate in the number of outstanding geologists who have taken on the task of preparing textbooks on their subject To a remarkable degree a few textbooks of the first rank have contributed to the way geology has evolved, setting a pattern of thought which was to persist for several decades

Menard states in his preface that he is aiming this book at students who are not majoring in the earth sciences In other words this is a professional's textbook aimed at a wider audience and one which, in my opinion, may well become a guideline for those who are attempting to integrate various branches of the earth sciences using the term in its widest sense

Menard's approach is to provide readable syntheses on four or five fundamental divisions of each science, giving himself enough elbow room to treat the essentials but not so much as to become involved in detail In succession he takes up the subjects of the planet Earth, its deformation, the atmosphere and hydrosphere, and the manner in which these react with the solid rock, first to render it unstable and to weather it and then to produce the sediments Along the way he includes a brief section on volcanism and concludes with some 80 pages on water, fuels and mineral resources He follows Holmes rather than Lyell in treating the Earth as a machine and making this, not the stratigraphical record, the link-pin of his arguments I think he is particularly successful in conveying a sense of proportion and of the relative importance of phenomena When discussing the circulation of water, for example, he points out what a minute fraction is to be found at any one time in rivers and in the atmosphere, only 0 001% of all the Earth's water is in the air Menard describes the circulation of solid rock, of sediments, air and water as we understand these matters today His text would provide an excellent basis for any course dealing with the physical environment

The title indicates Menard's wider interests These emerge repeatedly through the book, but apart from the chapter on resources amounting to less than 10% of the whole, and the section on engineering problems caused by unstable ground, no extended portion of the text is devoted to them, though Menard does his best to emphasise their importance in the approach he adopts throughout the volume It is difficult to see how he could have done more He has been remarkably successful in his syntheses which in my mind constitute the main virtue of this book

J SUTTON

obituary

Alfred E. Mirsky

PROFESSOR ALFRED E MIRSKY, who first isolated and characterised genetic material from mammalian cells, died on June 19, 1974 He was 73

Born in New York, he gained his PhD from the University of Cambridge in 1926 and returned to the Rockefeller University in 1927 He became a professor there in 1954 and retired in 1971

Sir Frederick Brundrett

SIR FREDERICK BRUNDRETT, KCB, KBE, former Scientific Adviser to the Minister of Defence, died on August 1, 1974 He was 79

After attending the University of Cambridge, he joined the wireless branch of the Royal Naval Volunteer Reserve in 1916 He became Chief of the Royal Naval Scientific Service in 1946, and was appointed Deputy Scientific Adviser to the Minister of Defence in 1950 In 1954 he became Scientific Adviser and Chairman of the Defence Research Policy Committee

Gertrude E. Perlmann

PROFESSOR GERTRUDE E PERLMANN, an authority on protein chemistry, died on September 9, 1974 She was 62

After receiving her DSc, from the German University of Prague, she joined the Biological Institute of the Carlsberg Foundation She undertook research at the Harvard Medical School from 1939 to 1946, becoming a full professor in 1973 She was noted for her research on the structure of pepsin

Sir Francis Knowles

PROFESSOR SIR FRANCIS KNOWLES, Bt, FRS, the neuroendocrinologist, died on July 13, 1974 He was 59

Educated at the University of Oxford, he carried out pioneering work into the structure of neurosecretory systems In 1958, he went to the Department of Anatomy of the University of Birmingham, becoming Reader in 1963 and Professor of Comparative Endocrinology in 1967 Later that year he was appointed Professor of Anatomy at the University of London and Head of Anatomy at King's College, London

Announcements

Award

The **A T Shousha Foundation Medal and Prize for 1974** has been awarded posthumously to **Mohamed Taieb Hachicha**

Appointment

Piero Sensi has been elected president of the **Societa Italiana Scienze Farmaceutiche.**

Miscellaneous

The Harkness Fellowships for study and travel in the United States—1975 Candidacy is open to men and women (between 21 and 30 years of age on September 1, 1975) in any profession or field of study provided both their secondary and further education (or equivalent professional experience in lieu of further education) have been wholly or mainly in the United Kingdom For application forms or further information write to The Harkness Fellowships (UK), Harkness House, 38 Upper Brook Street, London W1Y 1PE

Gottlieb-Duttweiler Prize for the promotion of nutritional sciences Applications are invited from scientists with outstanding achievements in the field of nutrition research Applications should be sent by December 31, 1974 to Green Meadow Foundation, c/o Institute for Nutrition Research, Seestrasse 72, CH-8803 Ruschlikon, Switzerland

International meetings

October 24–26, **26th International Congress of Physiological Sciences,** New Delhi (Secretariat, 26th International Congress of Physiological Sciences, Department of Physiology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110016, India)

October 29–31, **5th International Symposium on Olfaction and Taste,** Parkville (Dr J P Coghlan, ISOT V Secretary, Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne, Parkville, Victoria 3052, Australia)

November 4–7, **9th International Symposium on Advances in Chromatography,** Houston (Albert Zlatkis, Chemistry Department, University of Houston, Houston, Texas 77004, USA)

November 6–8, **International Symposium on Pharmacokinetics and Drug Effects, Stockholm** (Hans H Linden, Director, Aputekarsocieten, Box 1136, S-111 81 Stockholm, Sweden)

November 7, **The Control of Cell Growth Regulation by Tumour-inducing Viruses,** The Royal Society (Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG)

November 10–14, **17th National Meeting, Academy of Pharmaceutical Sciences,** New Orleans (Academy of Pharmaceutical Sciences, A Ph A , 2215 Constitution Avenue, N W , Washington, DC 20037, USA)

November 23, **British Academy of Psychopharmacology Open Meeting,** London (Professor Max Hamilton, President, British Academy of Psychopharmacology, University of Leeds, Department of Psychiatry, 15 Hyde Terrace, Leeds LS2 9LT, UK)

Errata

In the article "Energy problems facing India" by N K Sehgal (*Nature,* **249,** 710, 1974) there were serious misplacings of the textual matter

(i) The last two paragraphs under "Genesis of problems" (page 711, starting with "A 'gobar-gas' ") should go at the end of "Meeting the challenge" (page 712)

(ii) The entire portion under "Fifth plan and beyond" starting with the fifth paragraph ("Petroleum products ") onwards, must come under "Genesis of problems" in place of the two paragraphs mentioned in (i) above

The corrected version is being published in *Scientific Opinion,* of which Dr Sehgal is the editor, in Vol 3, No 2

In the article "Antibody cell daughters can produce antibody of different specificities" by A J Cunningham and S A Fordham (*Nature,* **250,** 669, 1974) the first sentence of the legend to Fig 1 should read, "Photographs (a–c) and diagrammatic representation (d–f) of clear (c,f) sombrero (b,e) and partial (a,d) plaques "

Reports and publications

Great Britain

The Macaulay Institute for Soil Research Collected Papers, Vol 10, 1970/1972 Edited by A M B Geddes and R Noble Pp 26 + Collected Papers Nos 1-92 (Craigiebuckler, Aberdeen The Macaulay Institute for Soil Research, 1974) [127

Ordnance Survey Professional Papers, New Series No 26 The National Gravity Reference Net 1973 (NGRN 73) By D M Smith, P M Howell and A B D E Abernethy-Clark (Prepared in conjunction with the Institute of Geological Sciences) Pp 22 (Southampton Ordnance Survey of Great Britain 1974) £2 [157

Proceedings of the Royal Irish Academy Vol 74, Section A No 9 Anti-Compton Scattering By J L Synge Pp 67–72 18p No 10 Generalized Limits and Uniform Convexity By G de Barra Pp 73–78 15p No 11 A Minimisation Problem with a Solution of Spline Type By D W Arthur Pp 79–86 20p No 12 Poincaré Generators for the Free Spin 3/2 Field By G B Mainland Pp 87–90 14p Vol 74, Section B No 9 The Namurian of West County Clare By M H Rider Pp 125–142 34p No 10 Palynological Correlations in the Cork Beds (Upper Devonian-?Upper Carboniferous) of Southern Ireland By G Clayton, K Higgs, K J Gueinn and A Van Gelder Pp 145–156 + plates 3 and 4 38p No 11 Condensation of Aromatic Aldehydes with 5–Amino–4–Cyano–3–Cyanomethylpyrazoles By C N O'Callaghan Pp 157–160 14p No 12 A Late-Midlandian Section at Finglas River, near Waterville, Kerry Pp 161–178 28p No 13 A Tertiary Dyke System in South-West Ireland By P Morris Pp 179–184 15p (Dublin Royal Irish Academy, 1974) [157

Bulletin of the British Museum (Natural History) Geology Vol 25, No 1 The Ornithischian Dinosaur *Hypsilophodon* from the Wealden of the Isle of Wight By P M Galton Pp 1–152 + 2 plates £7 35 Vol 25, No 2 The Taxonomy and Morphology of *Puppigerus camperi* (Gray), an Eocene Sea-Turtle from Northern Europe By Richard Thomas Jones Moody Pp 153–186 + 8 plates £2 85 Zoology Vol 26, No 4 The Indigenous Earthworms (Megascolecidae Oligochaeta) of Tasmania By B G M Jamieson Pp 201–328 + 10 plates £6 75 Vol 26, No 5 The Freshwater Fishes of Rivers Mungo and Meme and Lakes Kotto, Mboandong and Soden, West Cameroon By E Trewavas Pp 329–419 + 5 plates £5 60 Vol 26, No 6 Notes on some Echinoderms from Southern Africa By A M Clark Pp 421–487 + 3 plates £3 40 (London British Museum (Natural History), 1974) [167

Energy and the Environment (Report of a Working Party set up jointly by Committee for Environmental Conservation, Royal Society of Arts, and the Institute of Fuel) Pp 45 (London The Royal Society of Arts, 1974) £3 [167

Sharks and Rays (Introductory Leaflet) Pp 6 (London British Museum (Natural History), 1974) 4p [177

Bulletin of the British Museum (Natural History) Entomology Vol 30, No 7 A Revision of the Genus *Passeromyia* Rhodhain and Villeneuve (Diptera Muscidae) By A C Pont Pp 339–372 £1 80 Zoology Vol 27, No 1 Anatomy of Head and Neck in the Huia (*Heteralocha acutirostris*) with Comparative Notes on Other Callaeidae By P J K Burton Pp 1–48 + 1 plate £2 30 (London British Museum (Natural History), 1974) [177

The Honeybee (Entomology Leaflet No 6) Pp 8 (London British Museum (Natural History), 1974) 7p [187

Other Countries

Consiglio Nazionale delle Ricerche, Laboratorio de Stude Sulla Ricerca e Sulla Documentazione Note di Bibliografia e di Documentazione Scientifica Scheme per una rete di Informatica nel Settore della Documentazione Scientifico-Tecnica Elementi Preliminari By Paolo Bisogno Pp 80 Indici Logica e Linguaggio Problemi di Catalogazione Semantica By Alfredo Serrai Pp 95 (Roma Consiglio Nazionale delle Ricerche, 1972 and 1974) [157

World Health Organization Technical Report/Series No 544 Uses of Epidemiology in Housing Programmes and in Planning Human Settlements—Report of a WHO Expert Committee on Housing and Health Pp 64 6 Sw francs No 545 Pesticide Residues in Food—Report of the 1973 Joint FAO/WHO Meeting Pp 42 5 Sw francs No 547 The Planning of Medical Education Programmes—Report of a WHO Expert Committee Pp 25 4 Sw francs (Geneva WHO, London HMSO, 1974) [157

Académie Royale de Belgique Mémoires de la Classe des Sciences 2e Serie, T XLI, Easc 2 l'Ostéologie d'*Elops* Linné, C , 1766 (Pisces Elopiformes) et son Intérêt Phylogénétique Par L Taverne Pp 96 (Bruxelles Académie Royale de Belgique, 1973) [157

USA National Institutes of Health Statistical Reference Book of International Activities, Fiscal Year 1973 Pp 52 (Bethesda, Md National Institutes of Health, Department of Health, Education and Welfare, 1974) [157

Journal of Carbohydrates-Nucleosides-Nucleotides, Vol 1, No 1, 1974 Pp 1–96 Edited by Robert E Harmon $60 per volume (6 issues), $4 50 for postage outside the USA and Canada (New York Marcel Dekker, Inc , 1974) [157

DFG 1973 Tatigkeitsbericht Deutsche Forschungsgemeinschaft Pp 274 Programme ubd Projekte Deutsche Forschungsgemeinschaft Pp 616 (Bonn-Bad Godesberg Deutsche Forschungsgemeinschaft, 1974) [167

Instituto Geografico e Cadastral, Lisboa Caderno Tecnico e de Informacao No 33 La Mesure de la Base-Etalon de Mata das Virtudes Par Dr Tauno Honkasalo Pp 44 (Lisboa Instituto Geografico e Cadastral, 1973) [177

United States Department of the Interior Geological Survey Professional Paper 839 Palynological Studies of the Coals of the Princess Reserve District in Northeastern Kentucky By Robert M Kosanke Pp iii + 22 + Plate 1 (Washington, DC Government Printing Office, 1973)$1 [177

Republic of South Africa Department of Industries Annual Report of the Division of Sea Fisheries for the Calendar Year 1970 Pp 25 (Sea Point, Cape Town Sea Fisheries Branch, 1974) [187

Records of the Australian Museum Vol 29, No 3 Spirorbinae (Polychaeta Serpulidae) from Southeastern Australia Notes on Their Taxonomy, Ecology, and Distribution By E W Knight-Jones, Phyllis Knight-Jones and L C Llewellyn Pp 107–151 $2 50 Vol 29, No 4 Descriptions of Four New Damselfishes (Pomacentridae) from Papua New Guinea and Eastern Australia By Gerald R Allen and D R Robertson Pp 153–167 + plates 1–5 $1 25 (Sydney The Australian Museum, 1974) [197

OECD Nuclear Energy Agency Second Activity Report Pp 71 (Paris OECD Nuclear Energy Agency, (1974) [197

Isotope Tracer Studies of Chemical Residues in Food and the Agricultural Environment (Panel Proceedings Series Pp 156 (Vienna International Atomic Energy Agency, London HMSO, 1974), 146 schillings, £3 20, $7 50 [247

United States Department of the Interior Geological Survey Professional Paper 802–B Summary of Turbulence Data from Rivers Conveyance Channels, and Laboratory Flumes By R S McQuivey Pp vi + 66 $1 55 Professional Paper 831–A History of Geological Investigations, Engineering Design, and Construction Methods of the Harold D Roberts Tunnel, Colorado By Ernest E Wahlstrom Pp iii + 13 $2 65 (Washington, DC Government Printing Office, 1973 and 1974) [247

Smithsonian Contributions to Zoology, No 158 Pelagic Studies of Seabirds in the Central and Eastern Pacific Ocean Edited by Warren B King Pp iv + 277 Washington, DC Smithsonian Institution Press, 1974 For sale by US Goverment Printing Office) $3 70 [257

Bulletin of the American Museum of Natural History, Vol 153, Article 2 Phylogeny and Zoogeography of Salmoniform Fishes and Relationships of *Lepidogalaxias salamandroides* By Donn Eric Rosen Pp 265–326 (New York American Museum of Natural History, 1974) $2 35 [297

Argonne National Laboratory 1973 Pp 32, (Argonne, Illinois Argonne National Laboratory 1974) [297

Changes in Stratigraphic Nomenclature by the U S Geological Survey, 1972 (Geological survey Bulletin 1394—A) By George V Cohee and Wilna B Wright Pp iv + 93 (U S Government Printing Office, 1973) [307

Mitsubishi–Kasei Institute of Life Sciences Annual Report 1973 (No 2) Pp 57 (Machida–shi, Mitsubishi–Kasei Institute of Life Sciences, 1974) [307

United States Department of the Interior Geological Survey Bulletin 1373 Geology of Gravina Island, Alaska By Henry C Berg Pp iv + 41 + plate 1 (Washington, DC Government Printing Office, 1973) $1 60 [317

Smithsonian Contributions to Zoology No 168 Ecology of the Squash and Gourd Bee, *Peponapis pruinosa*, on Cultivated Cucurbits in California (Hymenoptera Apoidea) By Paul D Hurd, jun, E Gorton Linsley and A E Michelbacher Pp iii + 17 60 cents No 169 Studies of Neotropical Caddisflies, XVIII New Species of Rhyacophilidae and Glossosomatidae (Trichoptera) By Oliver S Flint, jun Pp iv + 30 80 cents No 171 Spider Mites from Northwestern and North Central Mexico (Acarina Tetranychidae) By D M Tuttle, E W Baker and M Abbatiello Pp 18 60 cents (Washington, DC Smithsonian Institution Press, 1974 For sale by US Government Printing Office) [317

Mitteilungen aus der Biologischen Bundesanstalt fur Land- und Forstwirtschaft, Berlin–Dahlem Heft 158 Der Feuerbrand des Kernobstes, hervorgerufen von Erwinia amylovora (Burrill) *et al* Sammelreferat von Dr Wolfgang Zeller Pp 121 DM 15 Heft 159 Common Names von Schadgastropoden in 12 Sprachen Von Dr Dora Godan Pp 91 DM 10 (Berlin–Dahlem Biologischen Bundesanstalt fur Land- und Forstwirtschaft, 1974) [18

Annual Report of the Inter-American Tropical Tuna Commission 1973 Pp 150 (La Jolla, California Inter-American Tropical Tuna Commission, 1974) [28

nature

Volume 251 October 4, 1974

On goes the missile race

Whatever the international contortions of the last few years—the problems of the monetary system, the uncertainties about commodities, the disappearance of growth, the increased vulnerability of governments to non-democratic action—the business of defence has pursued its own self-effacing and growthful way. It is only the annual reviews provided by SIPRI and the International Institute for Strategic Studies (IISS) which remind us that those decisions taken 'with regret' a few years ago are now converted to expensive hardware for which there has been little public accountability.

The most recent occasion for us to consider defence expenditure and policy is the appearance of the annual report, *The Military Balance*, of the IISS. It arrives at a most appropriate time, for the failure of the Moscow visit of Mr Nixon to put any more significant teeth in arms control agreements between the superpowers can be assessed in terms of the now likely strategic development of the next few years.

The United States now spends 6.2% and the Soviet Union 5.4% of their Gross National Product on defence. Both percentages have been gently declining in recent years. In the American case, of course, the disengagement from Vietnam has at least checked the rise in military expenditure. In neither case does the strategic weapons programme seem to have been seriously hurt by falling budgets.

- In the United States, the contents of the thousand *Minuteman* silos permitted under SALT are changing rapidly, whilst the silos themselves are being increasingly hardened. Within a year all the 550 planned MIRV launchers will be installed, providing three independently targetable 200-kiloton warheads per launcher. The vast majority of the remainder of the sites already contain *Minuteman 2* vehicles which carry three warheads not independently targeted; there is nothing in SALT to stop the upgrading of all of these to MIRV capability.
- The Soviet Union is constrained to 1,618 silos and is now within 43 of that number. She can have 313 'heavy' missiles (with yields up to 25 megatons) and is close to that total. The remainder are at present mostly megaton SS-9 missiles, but MIRVing is on the way for these too.
- At sea, the United States is permitted 41 nuclear submarines with 710 missiles. At present half of the fleet is MIRVed (each missile containing at least ten 50-kiloton vehicles) but not all the rest will go this way, as ten Polaris boats will be pulled out of action in the late seventies and beyond, to be replaced by 24-tube *Tridents*. The warheads in *Trident* are MARVs—manoeuvrable re-entry vehicles, capable of manoeuvre after re-entry to avoid defences.
- The Soviet Navy is restricted to 62 'modern' submarines and is still 20 short of that figure, but building fast. As yet the 660 warheads carried are not multiple vehicles, but tests are under way.

The message is thus from all fronts that the qualitative revolution is now well advanced. Admittedly the Soviet Union seems to lag two or three years, but for both sides it is becoming plain that accuracy and sophistication is replacing numerical growth in the strategic game. Unfortunately since neither side is able, in its monitoring, to do much more than count devices, the prospects for verified arms limitations (and verifications is probably a *sine qua non*) diminish with time.

A case could be made that these qualitative improvements should be allowed to run their course. After all, if it will be possible to deliver nuclear warheads with an accuracy of a metre by 1990, as some believe, the payload could be reduced and the tradition that the military fights the military and does not draw in the general public might be re-established. There must always be the hope that war could be restricted to surgical strikes.

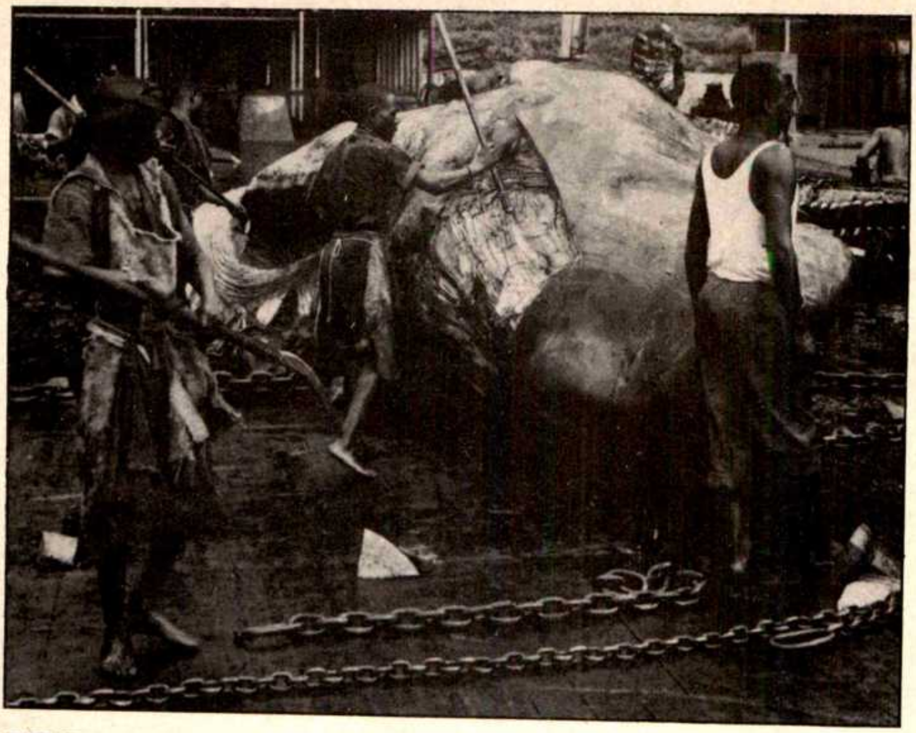
There are several reasons, however, why we cannot expect to have neat wars fought for us entirely by technology. An obvious one is that there are bound to be more than two participants in any future major war, and since the superpowers, in their pursuit of technological sophistication, have actively, if inadvertently, encouraged other countries to go nuclear but at a less sophisticated level, the multiplicity of nuclear-armed participants will inevitably confuse any bilateral game plan. But there is a more subtle reason why we should not believe that wars of opposing and highly sophisticated technologies will be more bearable for the civilian.

No nation would be satisfied that its future be decided entirely by surrogates. It is unlikely that the idea of a football match, say, between the superpowers—once the shape of the ball had been decided—would be deemed an acceptable way of settling political disputes. There has to be some personal involvement and sacrifice in war, and the growing trend away from this that we see in present and future strategic hardware poses an interesting question. If the early nuclear age has been marked by an uneasy truce between the superpowers because the consequences of major war were unspeakable, maybe the coming nuclear age will see further detente because a technologist's war between machines having reduced the unspeakable is politically unacceptable.

A hundred years ago

We referred some little time ago to the fact that a sum of about 30,000*l.* had been left to the " London Academy of Sciences." We hear that already several societies and institutions have sent in, or are thinking of sending in, claims. It is stated, however, that the Royal Society, which certainly is the nearest approach to the institution in Signor Ponti's mind, has not applied. The Royal Society is of course a mere private body, and might well be held to be justified in refusing to incur the responsibility of distributing a large sum for the furtherance of science; but the miserable chaos in our scientific arrangements is none the less strongly brought out by the present juncture. In England, truly, Science is a body without a head !

From *Nature*, **10**, 470, October 8, 1874.



Whales: conserving a resource

In spite of an expression of 'cautious optimism' after this year's meeting of the International Whaling Commission, and a recent Nature article which was optimistic about whale populations, there are still fears that all is not well in the whaling industry. Dr Sidney Holt, Director of the International Ocean Institute in Malta highlights some of the problems still confronting whale conservation and management.

AT the end of the meeting of the International Whaling Commission last June, a number of interested non-governmental organisations put out a statement expressing "cautious optimism" regarding the results achieved there. Dr Ray Gambell has been commendably prompt in bringing these results to the attention of readers of *Nature* (August 9, 1974). We who participated in the IWC meeting must agree that the adoption of what Dr Gambell calls the "amended moratorium proposal" was a significant advance towards rational management of whaling. This is, however, no time for complacency by scientists, governments or the interested public, and Dr Gambell's article is not, I think, sufficiently critical of the present state of whale population assessments. It is not insignificant that Dr Gambell illustrated his article with hypothetical diagrams rather than with an example. "The exact forms of the relationships . . . are not known for certain . . ." is far too mild an expression of real doubts. A number of weaknesses in present calculations are well known to members of the IWC's Scientific Committee, although there seems to be less divergence of opinion within the Committee than in the past. I have drawn the Committee's attention to other deficiencies identified by a special working party of the FAO Advisory Committee on Marine Resources Research with which Dr Gambell is also associated. I will not reiterate these but merely observe that although they do not all point in the same direction, their general sense is that the IWC's Scientific Advisers may still be erring on the side of optimism. Here, however, I wish to draw attention to some specific points raised by Dr Gambell's article. There is a slip in his exposition of the simple theory of density-dependent rates. The natural mortality rate might "fall as whales are caught", but not because they "are caught before they have a chance to die naturally". In all models used the mortality rates, both "natural" and "due to whaling" are, by definition, independent exponential coefficients, not absolute quantities.

Then there are two significant errors in the table of present sustainable yields (SY) given. SY for female sperm whales in the Southern hemisphere is 1,800, not 4,500. The latter figure is the calculated maximum sustainable yield of females which could be obtained if their numbers were reduced. This would, however, be, in part at least, at the expense of the catch of males, which are far more important because they are both bigger and more numerous. The high quota of 5,000 is designed to reduce the number of females quite drastically—an act of doubtful wisdom. The SY of 1,700 given in the table for North Pacific female sperm whales is correct (insofar as any of these calculated values are "correct"); the corresponding MSY is 3,400 which is considerably less than the permitted quota of 4,000.

The second error concerns the Minke whale. The stated present SY in the Antarctic of 7,000–12,000 is an estimate of the maximum SY; the Minke is said to be very lightly exploited at present and its present SY is certainly much lower than 7,000. That, however, is not the main point. There are practically no hard facts about the population of this species. As the report says, "The Scientific Committee was unable to reach any conclusion . . . for Minke whales," and it " advised caution in setting catch limits . . ." By analogy with other species, which are rather better known, the Scientific Committee proposed a quota of 5,000, as they did last year. This was not acceptable to Japan and the Soviet Union, who share about equally the present catches, and the 7,000 quota was a compromise; it cannot be described as the outcome of "rational policy". The case of the Minke is important because it has suddenly become, in the last two seasons, an important item in the catches of baleen whales by the pelagic expeditions. From being negligible in the 1968–69 season, it jumped to 12% by weight in 1969–70, and grew to nearly one-third of the total of 190,000 tons taken in the 1973–74 season. The stepping up of the Minke catch is one more step (the last?) in the process of maintaining the profitability of Antarctic whaling by successively exploiting smaller and smaller species of whales—the earlier steps were from blue whales to fin whales to sei whales. That this process is now regulated—but hardly restrained—under a quota system does not mean that it is "rational", that it will ensure survival of a viable population of Minke whales, or that it will necessarily ensure the possibility of high sustained yields from that species in the long term. Judging from past performance, there may be a lapse of up to ten years before the member countries of the IWC are able to agree to act on important advice from its Scientific Committee. Some of the international commissions which regulate the sea fisheries have a similar concept of time and urgency but biologically fishes are more resilient than marine mammals.

It is also a general feature of the history of whaling that by the time there is widespread concern about the survival of one species the industry has already moved to heavy exploitation of another. Thus, while in the past decade or so, "conservation minded" organisations have become aware of what was happening to the blue whale and, lately, the fin whale, the sperm whale has become by far the most important species both in the Antarctic and in world catches. It now accounts for over 40% by weight of the total catch of all species of whales. Its monetary value is not quite so high because, although sperm oil, the main product, fetches higher prices than, and has different uses from, the baleen oil, the sperm whale meat is not suitable for human consumption. (This means that the argument used by the Japan Whaling Association for continued intensive whaling because whale meat is an important ingredient of the diet of school children and poorer people is not applicable to the sperm whaling industry.) Scientific assessment of sperm whale stocks is especially difficult because of particular features of their biology and of the industry based on them. The IWC Scientific Committee believes that the level of catch of males is at about their maximum sustainable yield in numbers. It is said that a convenient aspect of sperm whaling is that the males are bigger than the females, there is much geographical segregation of the sexes, and the social behaviour (including a "harem" structure) are such that a large "surplus" of males can readily be caught. I find it difficult to reconcile this with the decision to reduce the size limit for females "in order to bring the sexes into better balance". Sperm whales are especially important to the Soviet Union, whose whalers now regularly take over half the world total, yet the USSR delegation to the IWC appeared to be having second thoughts about that decision.

But there is a further problem. Whereas males caught twenty years ago averaged 45 tons each in weight, now they average only 28 tons. The size of females has also declined (though not relatively so much because they have, until now, been less intensively exploited). In fact, the average size of all the large species of baleen whales also has declined over the period in which each of them has been intensively exploited. This means that management for maximum sustainable yield in weight (and hence maximum value of product) requires rather different criteria for management than are at present used. This is particularly true for sperm whales, but for all species the desirable level of population is higher than is needed to maximise the yield in numbers. This should please the whaling industry as well as 'conservationists'. The latter group wants survival of species and of stocks to be assured and for the whales to continue to play an important role in marine ecosystems. The former finds that exploiting stocks when they are at high levels of abundance is much more profitable than when the stocks are depleted. This is as true under management for sustained yields as it is for unrestrained whaling. The only trouble is that to maximise the weight yield from an overexploited stock, one has to agree to more stringent immediate conservation measures, and wait longer for their beneficial effects.

Incidentally, the fact that a substantial part of the sperm whale catch is by ships flying flags of non-member countries of the IWC, that it is not unknown for industries to operate under a convenient flag, especially when international regulations become burdensome, and that some countries refrain from joining the IWC for political rather than technical reasons, suggest that it is desirable for the United Nations Environment Programme and other UN Agencies with wide membership to retain their interest in whaling, while continuing to assist and support the IWC.

To maximise the weight yield from an overexploited stock one has to agree to more stringent immediate conservation measures and wait longer for their beneficial effects. . .

Dr Gambell's article gives the impression that defining MSY in terms of weight involves a new concept requiring special study. On the contrary, apart from being practically universal in fishery management, the MSY (weight) criterion was introduced to the IWC over 13 years ago, at the same time as the attainment of sustainable yields in numerical terms was first seriously discussed there. The necessary data are available, as are the mathematical models; it is simply a question of feeding one into the other. The whaling industry has naturally always measured its success by the weight and price of its products, not by the number of whales caught, and it is time the scientific advisers to IWC did the same.

Calculations of recent and expected yields in weight show that if the 1974–75 quotas for pelagic whaling in the Southern hemisphere are reached (as the 1973–74 ones were not) the catch of all species next season may reach 380 thousand tons, 2 or 3% more than in 1973–74. This pelagic industry is still important; in recent years it has accounted for 60% of the world catch of the larger whale species.

Dr Gambell is right in saying that more research is needed—and at practically every level, including critical study of the mathematical models currently used, and investigations of the ecological interactions between the species of whales, and between them and other marine organisms on which they feed or with which they compete. In this connection it should be noted that the suggestion to which Dr Gambell refers in his opening paragraph—and it was no more than a hypothetical suggestion—that catching some species may help the recovery of others, is based solely on the fact that they overlap in the composition of their diet. No quantitative evidence whatever has yet been presented to support it. In fact, a number of important assumptions that are regularly made have not yet been tested; these include the assumptions that each whale population is in a steady state before man starts exploiting it, and that his effect on it is merely reversed if and when his predation is eased.

Meanwhile, the world catch of whales is, despite the depletion of the resource and because of the rising prices of meat and oil, still worth about £80 million annually. It could, if stocks were permitted to recover, be worth many times that at present prices—and even more with the expected relative rise in the prices of animal protein in the coming years. Against this we recognise that Dr Gambell and his few colleagues work with very limited facilities and funds, as do similar groups in other countries. Although no whaling expeditions now fly the British flag, the United Kingdom is stil a considerable importer of some whale products, and presumably retains a long term interest in resources which could once again yield 10% or more of the total value of the world marine harvest, and practically all from the area of ocean which will be beyond national jurisdictions even if the United Nations Conference on the Law of the Sea agrees eventually to an "economic resource zone" 200 miles wide. Now is the time to make substantially greater means available for research on cetaceans and for international management of them. It would be folly, as some seem to wish, for research to be cut back because of the decline of an important industry, which is a result of our past failure to protect the resource base. At the same time it must be hoped that research on the use of the other living resources of the open ocean, such as the Antarctic krill and the deep water cephalopods, will not be left entirely to the two nations remaining in the business of pelagic whaling. If this is too big an enterprise for other maritime countries, should we not be thinking of a substantial international programme of research and development? •

SCIENCE COLLEGE

international news

US plans for solar energy

by Colin Norman, Washington

THE US Congress is now putting the finishing touches to a bill which will greatly expand research and development activities aimed at converting solar energy to practical use. Among the provisions contained in the legislation are the establishment of a Solar Energy Research Institute, coördination of solar energy programmes which are now spread over several agencies of the federal government, and the promise of as much as $1,000 million over the next five years for research and development.

Both the Senate and the House of Representatives have now passed slightly differing versions of the bill, and a House-Senate conference committee was appointed last week to work out a compromise. According to congressional staff members, there should be little trouble reaching agreement, and Congress should be able to send a bill to President Ford to sign before the session ends.

The bill reflects the feeling on Capitol Hill that solar energy has been given too low a priority in the Administration's energy planning, and that present efforts in harnessing sunlight for practical purposes have been too fragmented. It also reflects the fact that a vote for solar energy goes down well back home in election year.

The House and Senate versions of the bill are in complete accord on two major points. First, they would both establish a Solar Energy Coordination and Management Project, consisting of top officials of five governmental agencies and one Presidential appointee, to oversee the federal government's solar energy activities. Such a body "represents a recognition that changes need to be made in the way in which priorities for energy research and development are set and the way in which the Federal Government organises itself to carry them out", according to one of the bill's chief sponsors, Representative Mike McCormack of Washington State. At present, the National Science Foundation has been assigned the leading role in solar energy research and development, but at least a half a dozen agencies have a finger in the pie.

The second chief point of agreement is the need to establish a special Solar Energy Research Institute. Modelled on one of the laboratories of NASA or the Atomic Energy Commission, the institute would be established at either a new or an existing energy laboratory of the federal government. No determination has yet been made on where the laboratory should be established, but Congressional jockeying can be expected from politicians who would like to have it in their home state.

Both bills also define solar energy to include not only direct conversion of sunlight to electrical power and heating, but also ocean thermal power conversion and windpower.

The chief difference in the bills is the amount of money which they recommend should be spent. A preamble to the Senate version states that solar energy research should be funded to the tune of $1,000 million over the next five years, and it also provides $100 million for the remaining half of the 1975 fiscal year. The House version, on the other hand, would provide only $2 million this year to draw up a research and development, and whatever is necessary in following years. McCormack said during hearings on the Senate bill, however, that the $200 million a year estimate is probably "in the right ballpark". Whatever the conference committee finally decides, a large increase from the present level of $50 million a year can be anticipated.□

Research in France: austerity budget

from the Staff of La Recherche

WHEN President Pompidou arrived at the Elysee in 1969, he set out to reorganise the structures of scientific policy. The creation of a Ministry of Industrial and Scientific Development in 1969 actually marked a kind of break with the Gaullist period of scientific policy, even though research, for two years, had no longer been given the priority that it had after 1958. This Ministry, which inherited projects from the former Ministry of Industry and those of the former Ministry of Research also had to oversee the Deligation Genèral à la recherche scientifique et technique (DGRST) which is (or should be) in charge of the coordination of science policy.

It is this hybrid and rather awkwardly functioning ministerial structure that M. Giscard d'Estaing inherited last May. When the new government was set up, Prime Minister J. Chirac decided not to modify the structures of scientific policy fundamentally and entrusted the responsibility of a Ministry of Industry and Research (whose projects are almost identical to those of the ex-Ministry of Industrial and Scientific Development) to M. d'Onano. Hhe first problem which confronted the new minister was the preparation of the budget for 1975. Since it was imperative to fight inflation, drastic cuts in public spending for the following year had to be imposed and thus scientific research will have an austerity budget in 1975. After the Prime Minister's bargaining session, the increase in total funds for research and development included in the 'Enveloppe Recherche' (which comprises the largest part of civil research except for telecommunications and major civil aeronautical programmes) will be about 13% in 1975. This budget raise in funds is approximately as large as that planned for all state expenses, but it may be insufficient to compensate for inflation which is currently increasing at an annual rate of about 15%.

Of course, this budgetary growth conceals serious disparities. First of all, one must realise that basic research will be treated to a relatively lesser degree in 1975 than in 1974 since the increase of programme authorisations for the CNRS will be about 7% next year and this agency will be granted very few newly created budgetary positions for researchers: about 100. Only medical research will continue to have relative priority since funds earmarked for INSERM (Institut National de la Santé et de la Recherche Medicale) for equipping its laboratories will be increased by 28% next year. On the other hand the big projects (the so-called 'grands programmes') which have been the object of much controversy for several years in France will undergo an austerity squeeze to a varying extent. Thus, although the budget increase for the CEA (Atomic Energy) is about 16%, that for CNES (Space Research) investments is practically nothing, as are the funds for the 'Plan Calcul', that is computers.

At any rate, in the fields of space and computer science, budgetary decisions are perhaps only temporary. The choices made reflect a certain agreement to make research in the field of energy a high priority item (funds will be increased by 27%). As far as aid allotted to industrial research is concerned, the 1975 budget will mark a slight acceleration in growth (a 16% increase for the process of aid to development). This research budget, the first of M. Giscard d'Estaing's seven years' term gives some indication of a trend but it does not constitute in itself a scientific policy. The reorientation of research efforts in relation to new priorities implies structural reforms. On this level, major decisions are still being studied. A reorganisation of the Ministry of Industry and Research and of the DGRST could take place in the autumn. The only measure taken in this regard during the summer was to connect the "Delegation à l'informatique" which oversees the 'Plan Calcul' for computers to a new Department of Electronic Industries and Computers within the Ministry. But, at present, the new government's most important innovation is the creation of a Secretary of State to the Universities who is completely autonomous and oversees the universities and the CNRS, thus restricting the power of the Ministry of National Education to elementary and secondary schools. This secretary is expected to outline a policy for university research which the former Ministry of National Education never really concentrated upon. Only during the next few months will one really be able to judge the objectives that the new government intends to pursue in scientific policy. □

Business: problems for Ferranti

from Roger Woodham

ANOTHER British company, the electrical and electronics group Ferranti which makes a wide range of things from naval and aviation electronic equipment to the humble domestic electricity meter, is short of money and has persuaded the government to help out. Looking on the gloomy side and assuming that Ferranti's financial needs continue to grow unabated in the near future, the government has bought the company only a few months of respite with its promise of guarantees to the tune of £5 million so that Ferranti's bank overdraft can be increased yet again. These are the stark facts that make estimates that British industry will be short of perhaps £3,000 million by the end of the year seem plausible.

Ferranti is an interesting case inasfar as much of its work is inextricably linked up with European defence. Sales last year were worth £72 million, £52 million of that in the British market with 40% being accounted for by defence contracts with the British government. Many of its exports go to Europe but the company describes the destinations of its foreign sales as "traditionally erratic", citing a large one-off contract to supply equipment to the Brazilian navy last year as an example. In spite of all this, profits have been hard to come by in recent years, for the company as a whole at least; a profit of £3 million in 1967 turned gradually into a loss of £900,000 in 1971 and the accounts are still in the red after two years of improvement.

The development budget—Ferranti says it does no 'research' in the accepted sense—runs at £15 million a year, about a third of which is for governments. The company declines to say how many of its workforce of 17,000 are involved in development because a correlation of money and heads" could be commercially embarrassing".

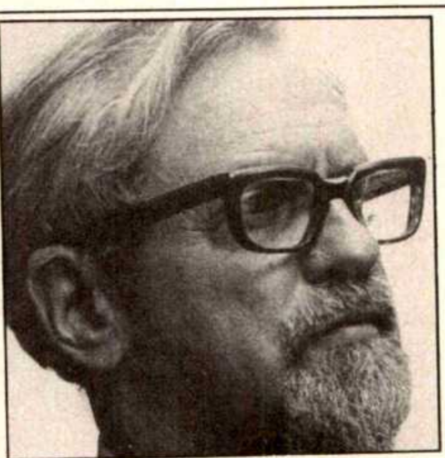
The future for Ferranti in the longer term will depend to some extent on who wins the general election. The present government has several options:

- To buy a stake in the company, thus wresting control from the Ferranti family.
- To bring in an outside organisation to manage the whole concern.
- To provide, as part of a more wide ranging industrial policy, some form of financing for the medium term.
- To persuade the company to sell off parts of itself in order to create a more viable unit. There are several precedents, for example, the sale of Ferranti computer interests to what is now the computer company ICL in the 1960s. □

AMERICA'S first scientifically-based Center for UFO Studies, established by J. Allen Hynek (right) after a wave of UFO reports across the States last year, is planning to ask the National Science Foundation and NASA for funds in the near future. The Center, a loose affiliation of scientists from a dozen US universities, is currently funded by private donations, but feels that its stock in trade—scientific credibility—stands sufficiently high for approaches to be made to establishment bodies like NSF and NASA.

Hynek, professor of astronomy at Northwestern University, Illinois, and author of the best-selling *The UFO Experience*, has been interested in unidentified flying objects for years, and has developed contacts with what he calls the "invisible college" of qualified scientists who believe the UFO phenomenon to be worthy of investigation. In correspondence they agreed that it was time to set up a Center to provide reliable UFO information and to collect reports of events for investigation.

Hynek rented the services of a toll-free telephone switchboard that is manned round the clock, seven days a week, and distributed its number to police throughout the United States. UFO reports on this hot line average about one a day (or night). Depending on their importance, cases are followed up in person or by a questionnaire.



The scientists of the Center are preparing a report on the 1973 UFO wave in which 1,500 cases are listed. The sightings started in the south-eastern states and, in Hynek's words, "spread along the river valleys". When published next spring, the report will be a valuable study of a major UFO event.

Hynek's work has started a steady shift in public and scientific attitudes to UFOs which are documented in a revealing hour-long TV programme being screened coast-to-coast in December by NBC. "I think the programme's impact will be considerable," says Hynek. "When the next wave occurs the Center will be there to serve."

Hynek is impressed by the fact that the nature of the phenomenon itself is the one thing that has not changed. "The typical UFO report today is essentially the same as it was 10 years ago," he says. Yet he carefully points out that he does not support the idea that UFOs are nuts-and-bolts spacecraft from other worlds: "There are too many things against it. It seems ridiculous that any intelligence would come from such great distances to do reportedly stupid things like stopping cars and frightening people. And there are far, far too many reports."

For Hynek and the members of his "invisible college" there remains no doubt that the UFO phenomenon is real. But its explanation? He draws a parallel with early attempts at explaining what makes the Sun shine, which faltered through lack of knowledge of physics. Currently, all the UFO Center can do is to define the limits of the problem. Says Hynek: "We're setting down the things that any hypothesis will have to explain." And, returning to the conclusion of *The UFO Experience*, he adds: "When the solution does come, it's going to be one hell of a quantum jump."

IAN RIDPATH

End of MRC's Edinburgh unit

by Peter Newmark

AFTER seventeen years of pre-eminence in a much-vaunted field of science the Medical Research Council (MRC) Molecular Genetics Unit closed at the end of September. Set up in 1957 at the Royal Postgraduate Medical School, Hammersmith Hospital under the directorship of Bill Hayes, the unit was originally known as the Microbial Genetics Research Unit. In the early 1960s a desire to become more closely involved with teaching as well as research led to negotiations with Edinburgh University. This culminated in a package deal whereby Edinburgh set up a department of molecular biology in which the MRC unit was also to be housed. The chair of Molecular Biology was given to Martin Pollock and his department started up in 1965.

The MRC unit moved up to Edinburgh in 1968 but it was not until 1970 that the department as a whole felt itself to be fully settled down. It was taken for granted at the time of the move that the MRC unit would survive at least until 1978 when Professor Hayes would be 65 and therefore obliged to retire as director of the unit. However in 1971 Professor Hayes began to consider an early departure. It was this that finally precipitated the premature closure of the unit this year.

When the unit was founded microbial genetics was a relatively young subject, but it was clear that it was due for rapid expansion. As such it was an ideal candidate for the MRC to support, in that one of their prime justifications for providing long-term support for a subject is when "for reasons of scale, subject specialisation and duration (it) could not as effectively be organised within a university or medical school". Few people, at least in retrospect, doubt the prudence of the MRC in injecting funds into microbial genetics at that time. The unit rapidly became pre-eminent and began to attract visiting researchers from many countries. It consistently produced very high quality research into the bacterial mechanisms of, for example, chromosome and plasmid replication, transmission of drug-resistance, cell division, host restriction and the control of biosynthetic pathways. It also, in time, achieved a fine record of dissemination of its research interests into British universities. Former members of the unit took up chairs at Kent, Leicester, Newcastle and Sussex.

There is no doubt that the high standards of research emanating from the unit have continued up to the present time. A fall-off in the quality of the unit's research was therefore not the reason for premature closure. It is, however, hard to define exactly what the reasons were, since it is not MRC policy to divulge them except in very general terms.

The general MRC policy on units of this type is quite straightforward. A unit is set up to encourage a chosen subject on the initiative of an individual who then becomes its director. The planned tenure of the unit comes to the end with the retirement of the director at which time the future of the unit is reviewed. Usually the result is closure of the unit but, sometimes it is continued under a new director. In retrospect at least, it is now clear that the chances that the Molecular Genetics Unit would survive its director were already very slight in 1970/71. The MRC Annual Report for that year stated that "since genetics has now been developed considerably within the universities, the Council no longer feels that there is any general need to renew or establish units (in this field)." As a blanket statement this is not unreasonable. However it is not sufficient as a reason for closing the Molecular Genetics Unit, considering for example that the MRC saw fit to set up the Mammalian Genome Unit in 1973, also at Edinburgh University.

Two main interrelated factors must have been considered by the MRC in coming to their decision. The first was whether there had been a loss of momentum in the research being carried out by the unit. The second was the world-wide shift in emphasis in molecular genetics research from investigating the processes in prokaryotic organisms, especially bacteria, to eukaryotic organisms. The members of the unit dispute that there was a loss of momentum, though they admit that some degree of cohesion had been lost as their individual research interests diversified. They do not dispute that eukaryotic research has become very important, or that it is a suitable field for MRC support, but they are adamant that it would be a great mistake to think that bacterial systems have little left to offer to the advance of molecular genetics.

Although the unit's members are by now prepared to admit that its closure was not unreasonable they remain dissatisfied about the way in which this came about. When Professor Hayes in 1971 first considered retiring from the unit prematurely he immediately enquired of the MRC how this might affect the survival of the unit, at least until 1978. The initial MRC response appeared to be reassuring in hinting that the unit could continue. There was even talk of the likely candidates for taking over the directorship. Professor Hayes therefore settled on his move. Shortly after this and without any consultation with the members of the unit, came the news that a provisional MRC decision had been made to close the unit when the director departed.

As Professor Pollock, who acted as director of the unit after Professor Hayes' departure at the end of 1973, put it: "everyone was dumbfounded". He emphasises that everyone assumed the move to Edinburgh to imply continued long-term support from the MRC. And that had it occurred that this might not extend beyond five years many people, including himself, might not have been persuaded to make the move.

However, at least on paper, the 1971 decision was not specifically directed towards the unit, and, indeed, it heralded two years of discussions before being confirmed in mid 1973. Professor Pollock feels that (at least in the final stages) the handling of these discussions by the MRC was impeccable. Some members of the unit, whose futures were unclear for many months, are less complimentary, although nearly all are happy with the ultimate outcome. The discussions mainly revolved around the fate of the unit's staff. Three of its scientific members already had tenure with the MRC and were therefore at least assured of a job, although in principal they could have been requested to move to any MRC laboratory in the country. However, five senior scientific staff were left with an uncertain future. Professor Hayes decided to put these five up for tenure. The MRC may not have liked this at first but on consideration they encouraged it. Four of the five candidates were successful but again this assured only a salary, not a specified place to work.

The MRC at this stage hoped to disperse the tenured staff throughout the country, with the rationale that dissemination of expertise has a beneficial effect on its recipients. However, this proposal was met with great resistance on the grounds that the collective research capacities of the unit should not be disrupted and that too many personal hardships would be incurred by moving. It is possible that this resistance paid off. In the end, after complex negotiations, many of which involved discussions with the trade unions to which the unit's staff belonged, a solution was achieved. A solution which was unbelievably better than seemed possible in the early stages and which in many ways has the appearance of a face-saving operation by the MRC. All six tenured scientific staff members who wish to stay in the department are to be allowed indefinitely to remain there and do the same research as at present. They are to become members of the University Department of Molecular Biology, with their salaries paid for by the MRC.

In addition the MRC is providing reasonable technical assistance for

Letter from Italy (but only just)

By now everybody is aware that the Italian postal services are out of order The damage to the Italian economy must be enormous, if at all calculable· a typical "transforming" economy which is based on export-import and tourist trade cannot exist without communications Science, being deeply international by character, must suffer too

In fact, apart from the usual complaints about the scientific bureaucracy, the most popular topic of conversation among scientists is the abominable state of the postal services Everybody has a favourite story to tell about some postal disservice, which has been worse than anyone else's My worst case is the following an official letter was sent from an organisation to an individual in the same town, in fact the sender and the addressee lives 50 metres apart The letter took exactly 50 days to arrive. that is, one metre per day

Officially there is no strike—for that at least one could prepare better—but only a go-slow, so the results are completely unpredictable and haphazard, the fluctuations from the average delay are very large Apparently all this is caused by the unwillingness of the Ministry to give the men overtime There are delays the scientists cannot do anything about, and others about which they can For example, when ordering and receiving scientific materials, the orders go out with many weeks' delay (on top of the ordinary, "inevitable" administrative ones) and the stuff arrives with even greater delay (this is helped by the occasional wildcat strike of the customs) Not only is thus the experimental work greatly hampered, but in these inflationary times it causes unnecessary financial loss

Scientific journals arrive several months late, if at all (according to newspapers, many tonnes of printed material were simply destroyed by the agents to whom the post office entrusted them for distribution) There is no rational pattern about it In the Frascati science area there are several libraries subscribing to the same journals, however, a particular copy might arrive with several weeks' difference and in a random time-order The March 22 number of *Nature* arrived on the same date (July 12) as that of June 21 in one library, or the August 9 number arrived on the same date (September 2) as the August 23 in the next library, 100 metres across the road

So one is running around the various libraries to find the most up-to-date copy At least now the average delay is only a matter of a few weeks against several months not long ago Also one asks one's scientist friends abroad to photocopy a particularly important item and send it by registered express mail Perhaps it will arrive quicker that way (Not always in the spring more than a million registered letters accumulated in a special sorting office near Rome)

Of course, letters are late too—a request for a reprint posted in January in the States, arrived in July To send out a paper for publication by post is a game of chance So one tries to exploit people who travel abroad for missions, conferences In fact, the international organisations here, like FAO and ESRO, as well as large industries, have organised regular weekly courier services, *à là middle ages* Unfortunately this does not solve the problem of the incoming letters (unless they are addressed to a centre abroad and collected from there)

Particularly vulnerable are scientific meetings in Italy these days At a recent conference on the magnetosphere in Frascati ten scientists came to the conference whose registrations arrived from one week to three months after the event About 10% of the participants cancelled their registration, but their letters arrived when the conference was already over About another 10% did not come probably because of the postal difficulties One author who could not come sent his paper and his slides by post for presentation at the conference, they arrived one month after the conference Some of the main speakers did not receive the letter about the division of topics among them they got to know it for the first time from the printed posters!

Of course, the main message of all this was that 80% of the scientists came nevertheless, delivered their papers, discussed the magnetosphere and other problems while sipping a glass of good Frascati wine Thus the scientists are not completely downhearted They help themselves as they can They use telex for everything possible—or even impossible, like for instance, writing a common paper with colleagues in Boulder, Colorado, via telex Also, travel arrangements and other urgent matters they conduct over the phone Because of the numerous overseas calls, the telephone bill has gone up a bit Letters would be cheaper—but at least the telephone and telex still work For the moment George Magyar

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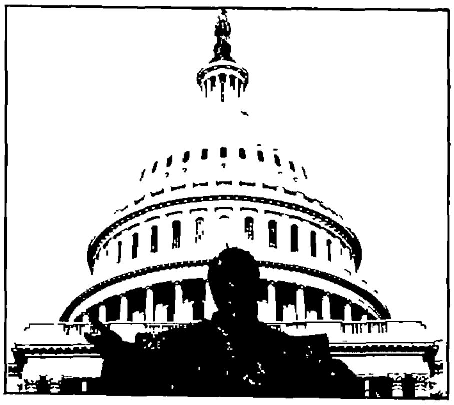
seven years This includes secondment of those members of the unit's technical staff who wish to retain the option of continued employment by the MRC Furthermore like any other research worker, the staff can now apply to the MRC for short-term support of graduate, post-doctorate and visiting research workers This they have already done with some success Ironically they might finish up with more MRC funds that the unit was consuming On the other hand the MRC has more direct control over what research projects they wish to support in the future

Are there any lessons to be learnt from the closing of this particular MRC unit? The final result is satisfactory in many ways but inevitably the interim two years generated a lot of anguish amongst those whose careers were threatened This has certainly confirmed a problem that the MRC were already aware of, namely that they must try and avoid supporting people for so long that it becomes unrealistic for them to find alternative jobs This has led to a continuing shift in MRC support away from long-term commitments

A more specific lesson that has been learnt is that consultation with a unit in future must precede provisional decisions about that unit With luck this may mean that future closures will involve less suffering For there is no doubt that two years of indecision has been detrimental both to the research and to the general morale of the ex-members of the late-lamented MRC Molecular Genetics Unit □



Washington seen

by Colin Norman

THE US Atomic Energy Commission has run into yet another snag with its liquid metal fast breeder reactor (LMFBR) programme The commission has now acknowledged what has been rumoured for some months—the estimated cost of building and operating a demonstration LMFBR has increased by 250% in two years The latest estimate, based on a year-long internal AEC study, is that the demonstration plant now being constructed in Tennessee will cost $1,736 million to build and operate for five years, in 1972, the AEC reckoned it would cost $699 million Furthermore, the AEC now says that the plant will not start operating until late 1982 or early 1983, three years behind the original schedule

These sharply increased estimates, which the commission puts down to inflation and anticipated construction delays, are already causing some government officials to question privately whether the programme should be allowed to proceed at breakneck speed although at present the LMFBR still ranks as top priority in the Administration's drive to make the United States energy self-sufficient The argument which is being increasingly raised against the LMFBR is that it is soaking up a huge proportion of the total expenditure on energy research and development, with virtually no short-term benefits In the last fiscal year, for example, LMFBR research accounted for more than $400 million in government funds, and according to estimates drawn up by the AEC's chairlady late last year, the entire breeder research programme (including the demonstration plant) could cost nearly $3,000 million over the next five years

The issue is likely to come to a head later this year when the AEC finally produces its much-delayed and completely rewritten final environmental impact statement on the LMFBR programme

● The American Association for the Advancement of Science (AAAS) has been forced to curtail a few activities during the summer in an effort to overcome a severe financial problem—the association ran up a deficit of more than $370,000 last year, and was probably heading for an even bigger loss this year Most conspicuous among the belt-tightening moves, which have been imposed by Acting AAAS Executive Officer Philip H Abelson, is a reduction in the number of pages contained in *Science*, and a decrease in the number of AAAS staff

Abelson, who took over as Acting Executive Officer of the Association early this summer when the previous occupant of that post, William Bevan, left to take up an academic appointment at Duke University, described the cut in the size of *Science* as a "painful expedient", but added that the journal "will not be permanently slim" He said that he hopes that it will be possible to wipe the deficit off the books by the end of the year

The chief reason for the financial crisis seems to be that the association expanded its activities during the past few years at a rate greater than its income allowed It has, for example, taken on a number of large programmes—such as an effort to improve the public understanding of science—which, although funded by grants, have resulted in large overhead costs to the AAAS In addition, it cost more than $150,000 to stage the AAAS annual meeting in Mexico City last year, and the whole situation has been exacerbated by a steep rise this year in printing and paper costs for *Science* Abelson said, however, that he does not expect that any major AAAS activities will be cancelled

● These are hard times for people who have a sweet tooth and a weight problem Not long ago they could sate their appetite for sugar with a choice of artificial sweeteners—cyclamates or saccharin—without risk of the sweetener turning straight to fat But cyclamates got banned for possibly causing cancer, saccharin stands accused of the same crime (although the jury is still out on that charge) and there is nothing to take their place

That is why G D Searle's new miracle sweetener Aspartame seemed for a moment to be a perfect answer to at least part of the sugar lover's calorie problem After all, the stuff breaks down into two essential amino acids in the body, it has proved to be safe when fed at moderate doses to rats and dogs and as recently as July it was approved by the Food and Drug Administration (FDA) for a limited number of uses (*Nature*, **250**, 259, 1974) What possible damage could it cause? Brain damage, according to one impassioned medical scientist

Dr John Olney, professor of psychiatry at Washington University School of Medicine in St Louis, has fired off a memorandum to FDA detailing his concern that Aspartame could pose a high risk to children because it may act synergistically with monosodium glutamate (MSG), a food additive which has been found to cause brain damage in mice

He has asked for a public hearing in which to state his case and the FDA has agreed, although no date has yet been set

Olney, who has for years been trying to bring public attention to the dangers posed by MSG, believes that the two additives could produce the same toxic effects, and he has some preliminary research results to bolster his case He argues that the safety margins calculated individually for MSG and Aspartame are both much lower than the FDA claims, and that if the two are taken together, there is no safety margin at all Furthermore, Olney takes issue with the FDA's contention that a person eating between 1 3 and 1 7 grams of Aspartame a day will be receiving only about 1% of the minimum dose found to be toxic to animals He points out that since the calculation is based on an adult body weight of 60 kg, a 15 kg child eating the same amount will not have an adequate margin of safety

Whether or not Olney's claims will be upheld by the public hearing, there is no doubt that the public relations aspects of his charges will seriously tarnish the image of Aspartame

Meanwhile, in a related development, the FDA refused last month to lift its ban on the marketing of cyclamates Abbott Laboratories, the sole manufacturer of the sweetener, had petitioned to get cyclamates back on the market, claiming that it has amassed sufficient data to prove that the chemical is safe and that the FDA's 1969 ban was ill-founded But the agency advised Abbott that its new data is inconclusive and insufficient

● According to the latest compilation of figures by the National Science Foundation, during the 1969–1975 fiscal years (in other words, during Mr Nixon's Presidency), the proportion of the federal science budget devoted to space activities declined dramatically

But the proportion spent on defence science and technology has remained constant At $10,217 million, the military research and development budget still accounts for more than half the total ☐

news and views

Blood disease caused by gene deletion

IN normal adult blood the major haemoglobin is a tetramer consisting of two α chains and two β chains In α thalassaemia, a disease prevalent particularly in south-east Asia, a deficiency in α chains results in the production of abnormal globins—before birth the tetramer γ_4 and in adult life the tetramer β_4

The genetics of α thalassaemia are not completely understood but at least two different mutations in allelic genes have been identified, designated α-thal 1 and α-thal 2 α-thal 1 results in a complete absence of α globin synthesis and in the homozygous condition results in Hb Bart's syndrome γ_4 tetramers are characteristic of this disease which results in intrauterine or early neonatal death In the heterozygous state, that is, when one gene has the α-thal 1 mutation and one has the α-thal 2, the disease is less severe so that survival into adult life is possible This condition is known as Hb-H and is characterised by the β_4 tetramer In some populations (including those in south-east Asia) there are two different structural genes for α globin The Hb Bart's syndrome follows the loss of all α loci, whereas in the Hb-H disease one out of four loci is functional, presumably allowing a low concentration of the normal $\alpha_2\beta_2$ tetramer

Weatherall and Clegg (*Br J Haemat*, **18**, 357, 1970) demonstrated that in victims of Hb Bart's syndrome the α chain is completely absent—there being no evidence of abnormal α chains Since then research interest has centred on the transcription of the messenger for α globin and its translation, to determine whether the α-thal 1 and α-thal 2 mutations reside in the α globin structural genes themselves or in other genes controlling the transcription of the structural genes or the translation of the gene product

Translation in a cell-free system of the globin messenger RNA isolated from reticulocytes of patients with Hb-H disease reproduces the protein synthesis pattern in the intact cell in that very little α globin is synthesised relative to β globin, suggesting that α globin mRNA is present in very small amounts of the reticulocytes The possibility that the cells contained normal amounts of a non-functional α globin messenger RNA could not however be excluded until the experiments by Housman and his colleagues (*Proc natn Acad Sci USA*, **70**, 1809, 1973) Using a probe for α mRNA (prepared by transcribing α globin mRNA into complementary (c) DNA with reverse transcriptase), they clearly demonstrated that the concentration of α mRNA was abnormally low in sufferers from Hb-H disease, and therefore most probably completely absent in Bart's syndrome

Having established that no messenger RNA for α globin was to be found in reticulocytes of α-thalassaemic patients, the next step in elucidating the molecular biology of the disease was to differentiate between the α structural genes and the regulatory genes controlling the transcription of the structural genes, as the site of the α-thal 1 mutation This has now been accomplished by two independent groups whose work is reported in this issue of *Nature* Ottolenghi *et al* (page 389) and Taylor *et al* (page 392) Their conclusions are in close agreement In the conclusive duplicated experiment a cDNA transcript of α messenger RNA was used to probe for complementary sequences in DNA isolated from normal human spleen The extent of annealing of the probe with normal DNA was about twice that observed for α thalassaemia DNA The small amount of hybridisation of the α cDNA to the α thalassaemia DNA may be due to contamination of the α mRNA with β mRNA so that the probe contains some β cDNA Another possibility is that the α cDNA is hybridising to the α-type gene zeta which codes for a globin produced at an early stage in foetal development Both groups conclude that in α-thal 1 homozygotes all (or a large part) of the structural genes for α globin are deleted from the genome This is the first time that a gene deletion has been shown to be the cause of a human disease

PAMELA HAMLYN

Lethal mutants and gene numbers

ONE of the most interesting discussions developing over the past few years has concerned the number of genes in the eukaryotic genome The apparent discrepancy between coding potential and probable gene number, about which this question is centred, has been most marked in *Drosophila* That one band might correspond to one gene was first suggested by Bridges[1] because of the apparent equivalence of the cytological and genetic maps of *D melanogaster* There are about 5,000 bands in the polytene chromosomes, yet the DNA content of *D melanogaster* could be divided into 140,000 lengths of 1,000 base pairs (a reasonable estimate for the average length of a gene) Even after restricting the capacity to code for proteins largely to the non-repetitive component of DNA, a concept supported by recent demonstrations that mRNA is derived largely or entirely from this class of sequence, the coding potential of *Drosophila* DNA remains in excess of 100,000 genes

Any estimate for the number of genes in *Drosophila* thus has immediate implications for the organisation of the chromosome At one extreme, if it is of the order of the number suggested by the coding potential of non-repetitive DNA (100,000), all of this component must represent structural genes and there must be many such genes in each band At the other extreme, a number close to that of the bands would support the earlier apparent equivalence but would bring into question the organisation of DNA within the band and the meaning of the 'gene' The experimental approach of the geneticist has been to accept the traditional definition of the gene as a complementation group and thus to attempt to define the number of such groups into which known mutants fall

Mutations in complementation groups must have visible phenotypes to be detected and recessive-lethal mutations are generally used in the genetic analysis of *Drosophila* In an analysis trying to achieve mutational saturation of the 3*A*1–3*C*2 region of the X chromosome of *D melanogaster*, which displays 15 polytene bands, Judd, Shen and Kaufman[2] identified 16 complementation groups, each of which was mapped with deletions to a single band (although with some ambiguity in region 3*B* where the bands are difficult to distinguish) In subsequent experiments to identify any additional classes of mutant that might have been missed originally, Judd and Young[3] isolated mutants that do not alter adult morphology or viability but cause delayed development or female sterility Two new complementation groups were identified by this analysis, both recessive female-steriles located in the 3*B* region The two groups are linked extremely tightly and may comprise a single unit that exhibits intracistronic complementation Another estimate has been made by Hochman[4,5], who has attempted to identify by lethal mutation every gene on the small fourth chromosome of *D melanogaster* This chromosome displays 50 polytene bands and thus corresponds to about 1% of the genome A complementation analysis of 200 mutations resolved 37 loci coding for essential products at which mutations are lethal or cause sterility when homozygous Another six loci had previously been identified through mutations that are recessive-visible, bringing the total to 43 Estimating the number of probable unmutated loci from the distribution of the 200 mutations in complementation groups suggested the presence of a further 17 genes, bring-the total to 60, slightly in excess of the band number The number of lethal complementation groups in the 3*A*1–3*C*2 X region is thus very close to the number of bands

Late lethal mutants of *Drosophila* can be identified by a technique developed by Shearn, Rice, Garen and Gehring[6] for screening third chromosome lethal mutants for those dying after the third larval instar—these mutants are normal in larval functions but defective in functions essential for pupal and adult development In a complementation analysis of three groups of third chromosome late lethal mutations (72 induced by EMS, 35 by nitrosoguanidine, and 35 by an acridine ICR-170), Shearn[7] resolved the first group into 60 loci, 55 represented by one mutant each, five identified by two mutants each, and one giving rise to seven mutants (presumably a mutational hot spot), the second group comprised 29 loci mutated once and three mutated twice, the third group constituting 31 loci mutated once and two mutated twice The Poisson distribution provides a formula which states the relative frequencies of complementation groups not mutated, mutated once, mutated twice, and so on By fitting the experimental data (ignoring the hot spot) to a Poisson distribution for each of the three sets of induced mutants, the total number of third chromosome genes able to give late lethal mutants was estimated as 364, 172 and 273 respectively Since 23% of all lethal mutants on the third chromosome are late lethals, this corresponds to a total of 750–1,600 lethal loci, the number of bands on the third chromosome is about 2.030

If it is true that each complementation group defines a single structural gene (plus any *cis*-dominant regulatory elements), the phenotype of different lethal mutants within each complementation group should be more or less uniform Shannon, Kaufman, Shen and Judd[8] concluded that their non-complementing mutants in the 3*A*1–3*C*2 X-chromosome region do indeed show similarities of phenotype Shearn has also found this to apply to the third chromosome late lethals, using a stringent test of uniformity in which imaginal disk developmental potential was assessed by dissection of disks, injection into metamorphosing hosts, and examination for differentiated structures Disk development is a good criterion since the disks, although superfluous for larval development, give rise to most of the adult structures, many or most late lethals are defective in disk functions (In an analysis of lethal mutants with imaginal disk defects, Shearn and Garen[9] recently identified 52 complementation groups on the third chromosome and concluded from a Poisson distribution analysis that there should be at least 435 such loci *in toto* on this chromosome, about a fifth of all complementation groups) Analysis of the imaginal disks of each pair of Shearn's non-complementing third chromosome late lethals suggested that in each case both mutants caused the same defect This is consistent with the idea that each complementation group represents a single gene function

A critical question which must be resolved before lethal complementation groups can be taken to identify all genes is whether 'null' loci exist—that is, genes whose failure to produce a protein leaves no identifiable mark on the phenotype Two extreme models are to suppose that null loci are atypical so that the small number of lethal loci identifies most genes, or that lethal loci are atypical and the genome largely constitutes a high number of unidentifiable null loci An alternative to these models has been discussed by Bishop[10] 'genes' might be multifunctional, each comprising a transcription unit coding for several proteins, so that although null loci might identify single dispensable protein chains in these units, mutation in other parts of the unit would be lethal

Some apparent null loci have been identified in *Drosophila*[11], but before these can be accepted as such it is necessary to show that mutation at each locus represents a deletion—otherwise it remains formally possible that some gene function remains, although in a form not detectable by simple assays for the protein And assuming that null loci exist, it becomes important to estimate their proportion relative to loci at which deletion is lethal in order to decide whether they represent a major class But for the presence of null loci to repudiate the complementation group–band equivalence by conferring a protein coding function upon most of the DNA in the *Drosophila* genome, it would be necessary for most genes to fall into this class, although it is clearly reasonable that the absence of some gene products might be compensated by alternative metabolic pathways, it is difficult to believe that most genes may be dispensable

It is of obvious interest to obtain estimates of gene numbers in other organisms as well as *Drosophila* and this adds to the importance of a genetic analysis of *Caenorhabditis elegans*, a nematode, reported recently by Brenner[12] *C elegans* is a hermaphrodite (each animal produces both sperm and eggs) and has about 600 somatic cells, half of which are neurones, making it suitable for a planned genetic dissection of the nervous system In an analysis of the DNA of this organism, Sulston and Brenner[13] show that the haploid content is 8×10^7 base pairs (about 20 times that of *Drosophila*) and 83% of the DNA sequences are non-repetitive To achieve a genetic analysis, Brenner has made use of the males which occur in small numbers in a culture and which have the chromosome constitution 5AA+XO instead of the hermaphrodite 5AA+XX

The 285 mutants, most recessive, induced by EMS and identified by morphological and behavioural differences from wild type, fall into six linkage groups and map at 82 genes The limited criteria used to identify mutants mean that only one class of genes is represented, but the large numbers of loci at which more than one mutation occurred suggest that mutational saturation of this class was achieved From the frequency of occurrence of these mutations, Brenner calculates that the average forward mutation rate induced by EMS under his conditions is about 5×10^{-4} per gene Since the non-repetitive DNA component of *C elegans* corresponds to about $6 7\times10^4$ lengths of 1,000 base pairs, if this mutation rate is typical of all genes it would imply that about 34 genes are mutated in each genome If all the DNA represented essential structural genes, each chromosome would thus carry six lethal mutations linked to the visible mutation isolated—clearly impossible since in this case it should not have been possible to isolate the visible mutant Another calculation is possible from the measured frequency of induced lethals per X chromosome, of 0 15 At an average induction rate per gene of 5×10^{-4}, this requires 300 genes This would mean that the six chromosomes constituting the genome contain less than 2,000 genes, representing only 3% of the coding potential Each of these 'genes' could contain 30,000 base pairs, a number similar to the average content of the *Drosophila* band It is interesting to note that the apparent number of lethal loci is of the same order of magnitude as the number of bacterial genes

Estimates of gene number have also been made for man Taking the frequency of deleterious mutation at each locus to be 10^{-5}–10^{-6} as suggested by Muller[14], the human genome of 3×10^6 potential 1,000-base genes should accumulate 3–30 harmful mutations each generation The unacceptability of such a high genetic load has led to suggestions that only a small part of human DNA codes for proteins By reversing the argument, if the actual rate of mutation is known, it becomes possible to calculate the number of genes, Ohno[15] has calculated a number of 4×10^4 and another estimate[16] is about 10^5 But these calculations involve assumptions about parameters such as generation times during evolution and rely upon indirect data for overall mutation rates Although they are therefore clearly not too reliable, the conclusion that only about 5% of human DNA should code for proteins is in line with the estimates for *Drosophila* and *C elegans*, if this number is correct, it does not, of course, prove the validity of the genetic calculations, but if much more human DNA codes for proteins, then the genetic estimates must be incorrect

Each lethal mutation formally identifies an essential site on DNA without making any implication about its function Some mutations may therefore lie in regulator loci rather than structural genes But since mutations within a gene or *cis*-dominant control element should fall into the same complementation group, the number of lethal complementation groups can be taken as the number of structural genes (Of course, any genes coding products which are regulators are included in this number together with genes coding enzymes or structural proteins) Accepting an estimate of 1,000 base pairs for the length of each gene, these low estimates for gene number imply that only a small proportion of eukaryotic DNA codes for proteins This conclusion is consistent with recent hybridisation analyses which show that mRNA is derived largely or entirely from non-repetitive sequences[17–19] and seems to be of comparatively low analytical complexity[20,21]

What can be the function of the non-coding DNA? In addition to all or almost all of the intermediately repetitive DNA component, for which a role in providing recognition elements in control systems can be proposed, this class includes most of the non-repetitive DNA component in which the structural genes are located One extreme model for the structure of the genome is to postulate that each identified lethal complementation group is of a small size, that is of the order of a 1,000 base pair coding sequence and an associated short control element, and that the remaining DNA is without any function identifiable by mutation, if this function is to code for proteins, then most gene products must be dispensable An alternative extreme model is to suppose that the genome is functionally divided into the small number of lethal complementation groups, each comprising some 30,000 base pairs and therefore including a coding sequence as only a minor part of its length, the purpose of the non-coding sequences can be a matter only for speculation The apparent correspondence between genetic loci and bands in *Drosophila,* however, which implies that a large amount of non-coding DNA is associated with the coding sequence in each band, fits well with the second model, and biochemical experiments on the sequences within bands therefore offer one of the most promising opportunities to resolve genetic organisation at the level of DNA

BENJAMIN LEWIN

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Nature and distance of the sources of Vela gamma rays

THE detection of bursts of cosmic gamma rays by instruments on the Vela satellites (Klebesadel, Strong and Olsen, *Astrophys J Lett*, **182**, L85, 1973) has given a much-needed boost to gamma ray astronomy More than twenty bursts have been observed and they have time scales ranging from one to thirty seconds with evidence of faster pulsations within each burst So far, no optical or radio emissions have been observed to coincide with the bursts

One of the fascinating aspects of a discovery such as this in modern astronomy is the speed at which the subject develops, into a few years is compressed what, in previous eras, would have taken decades or even centuries to accomplish The problem which is occupying the attention of many astronomers at the moment is the question of the distances to the sources of the bursts As always in astronomy this is a crucial question because, until it has been answered, we cannot estimate the scale of the energy release

One of the classic tests of the distances to a class of astronomical objects is the distribution of the objects over the sky This test relies on the fact that our Galaxy is a flattened distribution of stars and gas, thus, provided we can detect a large proportion of any class of objects in the Galaxy, these objects should seem to cluster around the Galactic plane On the other hand the universe outside the Galaxy seems to be isotropic, and extragalactic objects should be distributed isotropically over the sky One limitation of this test is that if the objects do lie in our Galaxy, but we can only detect the nearby ones, they will not seem to cluster around the Galactic plane, the naked eye stars are in a class of this type

An alternative method of gauging the distances to the sources is to measure the distribution in the strengths of the bursts as received at the Earth If the sources are distributed isotropically in space then the number of sources with an apparent strength greater than any value, *S*, will be proportional to $S^{-3/2}$ On the other hand if the sources are confined to the Galactic plane the distribution in the apparent strengths will be proportional to S^{-1}, if the sources are confined to a line as would be the case if they were predominantly in the local spiral arm of the Galaxy, the distribution in the apparent strengths will be proportional to $S^{-1/2}$

Strong and Klebesadel (page 396 of this issue) have applied these tests to the data they have received from the Vela satellites The information on the positions of the sources is poor because the angular response of the detectors on the satellites is isotropic, some information is available because each burst was detected on at least two well separated satellites and approximate positions can be derived from the difference in the arrival times of each burst at the two satellites The distribution in the apparent strengths of the sources seems to be proportional to $S^{-1/2}$ and Strong and Klebesadel claim that this suggests the sources lie in the local spiral arm of the Galaxy But to draw this distribution, they had to extend their observations to include a number of weak bursts and some suspicion must remain that their data on the weak bursts are not complete

Many experimentalists will view the conclusions of this paper with some concern The frequency of the bursts which have been observed by the Vela satellites is small, only ~20 bursts having been recorded in three years But the detectors on the Vela satellites were small and they were able to detect only the stronger bursts, there was hope that larger detectors flown on balloons would be capable of recording a much greater rate of events which would more than compensate for the shorter exposure provided by balloons If the distribution of apparent strengths varies as $S^{-3/2}$ then a large balloon-borne detector would record several events per day, but if the distribution varies as $S^{-1/2}$ the rate would be less than one per week and the experiment would be much less attractive

The lack of reliable distance estimates has not prevented theoreticians from speculating on the nature of the sources The short time scale of the bursts implies that the dimensions of the sources must be small, and most of the theories which have been proposed have assumed a stellar origin for the bursts Colgate (*Can J Phys*, **46**, S476, 1968) had predicted that a burst of X rays and gamma rays would be produced by the relativistic shell of material ejected from a supernova explosion, but, according to this theory, the time scale of the burst would be measured in microseconds and not seconds Moreover no supernova has been observed to coincide with a gamma ray burst Stecker and Frost (*Nature phys Sci*, **245**, 70, 1973) have remarked that the time structure of the bursts is very similar to that of the X rays which have been recorded from solar flares The energy output in a typical gamma ray burst, assuming the source is at a distance of 10 pc, is however ~10^{35} erg which is more than six orders of magnitude greater than that observed in the strongest solar flare Stecker and Strong suggest that the gamma ray bursts are flares on magnetic stars which have magnetic fields three orders of magnitude greater than that on the Sun and might therefore be expected to produce much more energetic flares But it is difficult to see why the time scales should be so similar when the magnetic field strengths are so different

Another theory, by Pacini and Ruderman, is published on page 399 of this issue This theory suggests that a burst is produced on the surface of a neutron star when the neutron star suffers a sudden increase in its angular velocity These sudden increases, or glitches, have been observed in a number of pulsars and it is thought that they are due to sudden decreases in the radii of the stars A change in the radius of a star would be accompanied by a change in the strength of its magnetic field, and there would be a large release of magnetic energy Some of this energy would be converted into relativistic electrons which would then radiate X rays and gamma rays Pacini and Ruderman estimate that there are enough pulsars within a distance of ten parsecs from the earth to explain the observed gamma ray bursts

Two papers in this issue report the measurement of gamma rays from pulsars Kniffen *et al* (page 397) have measured the gamma ray flux from NP 0532 and Albats *et al* (page 400) have observed gamma rays from PSR 0833-45 It is, perhaps, significant that all the well established sources of gamma rays, apart from the disk of the Galaxy, have intensities which show very rapid variations with time Bondi and others have stressed the potential which exists in astronomy in the study of phenomena on very short time scales, and it may be in this field that gamma ray astronomy will make its largest contribution

The stimulus provided in recent years by radio and X-ray astronomy has shown how much the scope of astronomy had been limited when measurements could only be made at optical frequencies, in the future we may see that the long reign of photographic techniques in observational astronomy, and the consequent long integration times of the observations, may have had an equally restricting influence on the development of the subject R R HILLIER

Historic first with community ecology

from Robert M May

A MAJOR purpose served by the First International Congress of Ecology, held in The Hague from 8-14 September was to bring to a focus some of the achievements of the International Biological Program (IBP) The motivating idea of IBP is that communities of plants and animals are productive systems with definable inputs and outputs The hope is that if we accumulate a sufficiently thorough body of measurement and knowledge about these inputs and outputs, we will end up with a better understanding of how ecosystems function, and, in particular, how they respond to the changes wrought by man Judged on their merits (rather than against the overblown aims which were often invoked to justify the early funding of IBP), the accomplishments are substantial

Consider, for example, the total annual production of plant material (primary production) of the earth's land masses Knowledge of quantities of this kind is clearly a *sine qua non* for rational management of the environment One of the earliest estimates of terrestrial primary productivity is due to Leibig around 1850 100×10^9 metric ton per year Ten years ago the widely accepted figure was $40–50 \times 10^9$ ton per year Drawing together many different strands of investigation, essentially all of IBP origin, H Leith of the University of North Carolina gave an estimate which is back near Leibig's guesstimate, namely $110–120 \times 10^9$ ton per year Leith emphasised that there are still uncertainties (another view, put by L E Rodin of the Komarov Botanical Institute, Lenigrad, and N I Bazilevich and N N Rozov of the Dokuchaev Soil Institute, Moscow, would assign a more generous figure of 170×10^9 ton per year), but the 110–120 figure seems now secure to within at worst a factor of two Around 1960 the total marine primary productivity was thought to be about 140×10^9 ton per year, Leith's and the Russian surveys agreed on a current estimate around $50–60 \times 10^9$ ton per year Thus over the past decade IBP studies have led to almost an order of magnitude revision in the terrestrial/marine primary productivity ratio

The major remaining uncertainties as to terrestrial primary productivity are in the tropics These equatorial regions, many of which are under increasing stress from the pressure of human activity, are in need of much further research Leith and others emphasised that temperate zone agricultural systems are unlikely to be best for tropical and subtropical regions

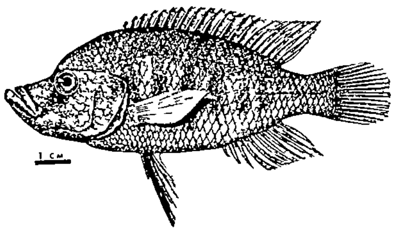
Reviews of current knowledge about secondary production—microbial and faunal production in terrestrial, freshwater and marine ecosystems—were given jointly by O W Heal from Merlewood Research Station, England, and S F MacLean, from the University of Alaska, and also by A Macfadyen, from the New University of Ulster These reviews, which included discussion of the comparative productive efficiencies of different classes of organism and of different levels in the trophic chain, were necessarily painted with a coarse brush, as comprehensive data are rare for most types of ecosystem and much of the IBP research remains to be digested Overall, there emerged estimates of the total secondary productivity in various types of ecosystem which seem secure to within a factor of ten In particular, there was general agreement that we are beginning to understand the limits to the rate at which material can be processed as it passes along the food chain, but there can be major upsets and fluctuations from year to year (as, for example, in pest outbreaks or lemming cycles)

One of the more noteworthy conclusions of the studies of secondary production is that the bulk of it takes place below the ground The birds and bees may engage scientific curiosity, but 90 to 99% of secondary production is by decomposer organisms in the soil and leaf litter MacLean humorously underlined this point by remarking that if humans must consume at the secondary rather than exclusively at the primary level (eat meat instead of only plants), they should at least consider eating earthworms rather than beef

In the plenary session devoted to "diversity, stability and maturity in natural ecosystems", G H Orians of the University of Washington, R H Whittaker of Cornell University and R M May of Princeton University marshalled various lines of empirical and theoretical evidence to suggest that communities with a rich array of species and a complex web of interactions (the tropical rainforest being the paradigm) are likely to be more fragile than relatively simple and robust temperate ecosystems This inverts the conventional wisdom that "complexity begets stability", but seems more in accord with such reported facts as (1) relatively simple arid ecosystems, including human populations with different economies, have persisted for long periods, fluctuating within well-defined limits (I Nor-Meir, Hebrew University, Jerusalem), (2) estuarine ecosystems seem to be disturbed less by, and to recover faster from, indignities imposed by man than do marine ecosystems of higher diversity (D F Boesch, Virginia Institute of Marine Science), (3) the tropical rainforest seems to be a 'nonrenewable source' when it is disturbed over sufficiently large areas (A Gomez-Pompa and C Vázquez-Yanes, Institute of Biology, UNAM, Mexico)

The final two days centred around "diversity, stability and maturity in ecosystems influenced by human activities", and "strategies for management of natural and man-made ecosystems" Here most speakers agreed that many aspects of human activities are now on a scale comparable with natural biogeochemical processes, but that both short and long term impacts are uncertain N Alexander of Cornell University discussed the response of microbial communities to human activities He pointed out that microorganisms are of enormous importance to the functioning of natural communities, that the impact of pesticides, polychlorinated biphenyls, acidic mine wastes and suchlike on various populations of microorganisms cause disturbances which range from "none to catastrophic", and that our ability to predict such outcomes are as yet woeful In a major review paper, J Jacobs of the University of Munich noted the ambiguities and uncertainties associated with the definition of human impact on natural systems, which are typified by a study of bird biomass and diversity in Finland in Helsinki, there were 21 species and a biomass of (in some appropriate units) 1,089, in rural areas near houses, an average of 80 species and a biomass of 371, in areas undisturbed by man, an average of 54 species and a biomass of 297

Indigenous fish



Haplochromis parorthostoma, found only in Lake Victoria, East Africa It is one of more than 100 endemic species of the cichlid genus *Haplochromis* which have evolved in the 750,000 years for which the lake has existed According to P H Greenwood (author of *Cichlid Fishes of Lake Victoria The Biology and Evolution of a Species Flock,* British Museum, London, 1974, £6 00) the explosive speciation was the result of a fortuitous combination of circumstances Lake Victoria (originally a group of lakes) offered many environmental niches, the extent of anatomical specialisation of the ancestral river fish was neither too great nor too small to allow further specialisation in Lake Victoria, and the complexity of the fishes' breeding behaviour allowed barriers to interspecific crosses to evolve

The above account touches upon some of the larger themes of the congress In so doing, it unfortunately has to pass over many interesting individual contributions I particularly enjoyed the account by M Cody, of UCLA, of the parallel and convergent evolution of the avifauna in 'Mediterranian' habitats in California, Chile, and Africa This study was part of the IBP Convergent Ecosystems Project, and it provided detailed illumination of the way species with different evolutionary histories come to have similar roles, to provide striking constancy in the ecological structure of geographically separated communities A diverse array of skills was brought to bear by G H Orians, R G Cates, D F Rhoades and J C Schultz, of the University of Washington, to elucidate some aspects of the cost–benefit accounting of the biochemical defences which plants maintain against the beasts which seek to eat them, and of the biochemical strategies in turn adopted by these consumers

I think the congress would have been improved by widening its scope in two respects

By focusing on the structure and function of whole communities of plants and animals (community ecology) For instance, theoretical notions about domains of stability, or about some numerical characterisation of ecosystem 'resilience', are beginning to be brought into confrontation with the real world in recent studies of low-dimensional systems such as insect host–parasite populations, for complex multispecies communities I think such theoretical speculations are a long way from quantitative testing against nature

Moreover, the congress was largely confined to technical aspects of how ecosystems work Given that the word 'ecology' has come to mean all things to all people, such a narrow construction was probably the wisest course for a professional meeting Even so, I would have liked to see one looser session, in which economists and professional ecologists aired speculations as to whether, for example, the contemporary global patterns of inflation are tied to real diminution in *per capita* supplies of food and energy, or whether these patterns are merely artifacts of present economic systems To have held such a session may have run the risk of vulgarising the congress, but to have done otherwise runs the larger risk of seeming to fiddle while the world burns

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Is the Martian core solid or liquid?

from Peter J Smith

FROM the mass, radius and moment of inertia of Mars as determined by orbiting and passing spacecraft, it may be reasonably concluded that the planet has a central core. But is the core solid or liquid? The correct answer to this question is not only of interest in its own right, it may also help to resolve a disagreement which is about to arise over whether or not Mars possesses an internal magnetic field. Last year, Dolginov *et al* (*J geophys. Res*, **78**, 4779, 1973) interpreted Mars 2 and Mars 3 magnetometer data in terms of just such a field, but apparently Bogdanov and Vaisberg will shortly argue that the observed magnetic field variations are the result of solar wind–planetary ionosphere interaction instead. Clearly, if it could be shown that the Martian core is solid, an intrinsic magnetic field (at least, produced by dynamo action) would be ruled out, although it must be admitted that proof of a liquid core would fail to resolve the issue either way.

In a situation where the confirmed presence or absence of an internal magnetic field cannot itself be used to infer the state of the core, the obvious approach is to examine the thermal conditions in the Martian interior. Unfortunately, there are no experimental thermal data from Mars. Mariner 9 revealed evidence of extensive volcanism (including a series of prominent shield volcanoes), mildly cratered plains similar to the lunar maria, and circular features such as domes and craters, but although this suggests that at some time in the past Mars must have been hot enough for partial melting to have occurred at depth, it can give no sure indication of present conditions.

In an attempt to deduce whether the Martian core is solid or liquid, Young and Schubert (*Geophys Res Lett*, **1**, 157, 1974) have therefore constructed a thermal model of Mars which involves no experimentally determined thermal boundary conditions but draws on experience and information gained from the application of similar techniques to the Earth. Indeed, as far as structure is concerned the model is a straight 'Earth model' with core, mantle and crust separated by rigid boundaries. The core itself is assumed to contain no heat sources (so there is zero heat flux at the core–mantle boundary), but heat sources are distributed uniformly throughout the rest of the planet (so temperature and thermal gradients are continuous across the crust–mantle boundary). The crust, whose outer boundary is taken to be an isothermal surface, is only able to conduct heat, but the mantle is a region in which solid-state thermal convection is an important mode of heat transfer. It is this last characteristic which particularly distinguishes the Young–Schubert model from most previous thermal model studies of planetary interiors.

In numerical terms, two sets of core parameters are examined: an iron core with a radius 0.4 of the total Martian radius and mass 0.1 of the Martian mass, and a pure FeS core of radius 0.6 and mass 0.26. These core models are the maximum density–minimum size and maximum size–minimum density cores consistent with observed mass, radius and moment of inertia of Mars. The radioactive heat source concentration in the crust–mantle is taken to be the same as that in the terrestrial mantle (2.61×10^{-7} erg cm^{-3} s). The thickness of the conducting crust will be determined by the depth at which the conduction solution for temperature rises to about 1,000° C above the surface temperature of the planet, and is thus highly dependent on the chosen value of heat source concentration. But crustal thickness should be at least 100 km because a thinner crust would probably be subject to plate tectonic activity, whereas no evidence of such activity is available from the Mariner 9 investigations.

The most important point to emerge from the model calculations is that the thermal convection in the mantle is remarkably successful in reducing temperatures (as in the Earth), thus vindicating its inclusion in the model in the first place. For a pure iron core, for example, a temperature difference of 8,000° C between the core–mantle boundary and the planetary surface when there is no convection is reduced to about 2,300° C when convection occurs at a Rayleigh number of 100 times the critical value for the onset of convection. The actual Rayleigh number is, of course, uncertain because viscosities are not known, but one certain point arising from the models is that, for pure conduction, temperatures rise above typical silicate melting temperatures. The implication here, therefore, is that convection in the Martian mantle is actually necessary to prevent large-scale melting there.

And because viscosities are unknown, it is not possible to deduce absolutely whether the Martian core is solid or liquid. But what can be said is that if viscosity is lower than $10^{22}-10^{23}$ cm^2 s^{-1} at temperatures above about 1,500° C, mantle convection will then be efficient enough to cool the core–mantle boundary to temperatures below those at which Fe or Fe–FeS cores would be liquid. As several workers have concluded that Martian viscosity is indeed below this limit, the Young–Schubert model suggests that a dynamo-produced magnetic field in the Martian core is not possible.

Finally, one other important conclusion to be drawn (specifically from the model temperature profiles) is that if partial melting occurs at all in the mantle it will do so at depths of about 300 km. By analogy with the Earth, this suggests that the Martian lithosphere would be several hundred kilometres thick, or intermediate in thickness between those of the Earth and the Moon.

Gravitational waves from collapsing stars

from P C W Davies

ALTHOUGH it is becoming increasingly likely that Professor Joseph Weber's longstanding attempt to detect gravitational waves from a cosmic source has not after all met with the spectacular success originally claimed, the stimulus that his work and propaganda has given to theoretical studies of this phenomenon continues to operate unabated. Gravitational waves are predicted to occur whenever massive objects are disturbed, much as electromagnetic radiation is produced by accelerating charged particles. The main difficulty in the detection of the former rather than the latter is the incredibly low cross section of ordinary matter to these disturbances. Consequently, only very massive and violent cosmic events occurring in relative proximity to the Earth are capable of producing a pulse of gravitational radiation sufficiently energetic to be detectable with current technology.

Attention has traditionally been given to three possible cosmic sources of such radiation: violent agitations occurring in the primaeval fireball of the big bang, objects crashing into black holes and the explosion or collapse of stars. Most books on gravitation contain order of magnitude estimates of the strength of the ensuing radiation, but few explicit numerical calculations seem to have been published. A welcome contribution to the physics of gravitational wave pulses from collapsing stars appears in the August 1 issue of *Astrophysical Journal* (**191**, L105–L107, 1974). Detailed calculations have been performed by Thuan and Ostriker of the Princeton University Observatory for a number of simple scenarios of the collapse of a white dwarf star into a neutron star or black hole.

It is well known that there can be no gravitational radiation from a spherically symmetrical collapsing object. A real star will, however, always possess asymmetries, and these tend to become amplified during the collapse process

The authors therefore consider a uniformly rotating star of uniform density and perform a calculation of the energy radiated away in the form of gravitational waves during the collapse to lowest order approximation in the gravitational potential This sort of collapse is known to occur for a white dwarf star with a mass a little in excess of the mass of the sun Calculations based on Newtonian theory show that a spheroidal shaped object (such as might be the shape of a rotating white dwarf) becomes more oblate as the collapse proceeds, until finally, after a finite period of time, it assumes the unlikely shape of a zero thickness pancake at a minimum radius, say r_{min} The Newtonian approximation will not be valid if r_{min} becomes comparable with the Schwarzschild radius r_{sch}

Thuan and Ostriker first find that a non-rotating spheroid of mass M which starts at rest at infinity with infinitesimal eccentricity, emits a pulse of gravitational radiation of total energy 37×10^{-3} $(r_{sch}/r_{min})^{7/2}Mc^2$ erg This result should be compared with the analogous case of two point masses collapsing in from infinity from an initial state of rest which liberates only $1\,2\times10^{-3}$ $(r_{sch}/r_{min})^{7/2}Mc^2$ erg Evidently spheroidal collapse is extremely efficient for the production of energy in this form

Next the authors include the effects of rotation The highest output would occur in this case if the star was very rapidly rotating with a large ratio of initial to final radius Under these circumstances the energy radiated is boosted further to 109×10^{-3} $(r_{sch}/r_{min})^{7/2}Mc^2$—a gain of 91 6 on the value for two collapsing point masses Moreover, as the authors point out, rapidly rotating stars are unstable to the growth of non-axisymmetrical perturbations, such as might be produced in reality by an oblique magnetic field These additional disturbances would increase the radiation emitted still further Physically, the reason why rapidly rotating collapsing stars radiate so much energy away in gravitational waves is partly due to the way in which the rotation delays the collapse of the equatorial parts of the star in a region of high gravitational potential during which time the poles quickly collapse inwards, so flattening the object still further

Turning their attention to a realistic model of a white dwarf, the authors then estimate the energy emitted during the collapse of a 1 4 $M_\odot$ Maclaurin spheroid with uniform rotation and uniform density of 10^9 g cm^{-3} An upper limit to this energy turns out to be about 10^{52} erg, and a lower limit around 10^{45} erg A single pulse of this sort will have Fourier components nearly constant over a wide frequency range, after which they fall rapidly

In practice a collapsing white dwarf might well emit a whole series of pulses as it 'bounced' back and forth through the pancake configuration, finally settling down into a neutron star However, assuming a Weber-type experiment attempts to detect a single pulse of the type considered, even under the most favourable circumstances the collapsing star would still need to be as close as 100 light years for any hope of success

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Still no gravitational radiation

by John Gribbin

LEVINE and Garwin report in the latest issue of *Physical Review Letters* (**33**, 794–797, 1974) a new negative result for the detection of gravitational radiation pulses, at a level six times more sensitive than the experiments they reported last year (*Phys Rev Lett*, **31**, 173–176, 1974) They say that they believe "the present results to be in substantial conflict with the detections reported by Weber"

The improved sensitivity over earlier results from the same group has been achieved chiefly through increasing the mass of the aluminium cylinder used as an antenna from 120 kg to 480 kg, the resonance frequency is now 1,637 Hz, close to that of Weber's apparatus, 1,661 Hz During the 27-day period December 3 to December 30, 1973, only one measurement out of $4\,1\times10^7$ values obtained for the mean oscillation energy kT contains a pulse substantially above the noise level

- According to Levine and Garwin, that pulse could be a gravity wave pulse, stress relaxation in the antenna, electrical pickup of "an intense but infrequent laboratory tone", or a signal-processor defect Since no coincident pulse was observed at the University of Rochester detector, the gravitational radiation explanation seems the least tenable

Further analysis of the data—including Weber's observations as far as they are available—seems to rule out both the possibility that gravity waves were detected in May 1973 but the pulses have not been produced since then, and the possibility that "hundreds of gravity waves were incident per day even during December 1973, giving our bar $<0\,3\ kT$" In addition, Levine and Garwin note that an error in the computer program used by Weber's group for some of the analysis would have produced a spuriously high number of pulses, and that Weber has acknowledged the existence of this error All in all, it seems that gravitational radiation has not yet been detected

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The changing micrometeoroid influx

from David W Hughes

THE disparity between the flux of micrometeorites at 1 AU obtained from lunar microcrater counts and from satellite data has been known for many years A typical example is shown on page 380, the satellite data coming from the work of Alexander (Baylor University, Waco, Texas), the data from the study of microcraters on the surface of lunar rocks and spherules coming from the work of Neukum and Schneider (Max-Planck-Institut fur Kernphysik, Heidelberg) In the 10^{-10}g region the two techniques give results which differ by a factor of 200 and it is of great importance to decide if this difference is due to some physical cause or is just an unresolved systematic error in one or both techniques Fortunately the influx of meteoroids to the lunar surface is the same as that to the Earth's upper atmosphere (most of the satellite observations are made in near-Earth space), the circumterrestrial dust cloud, thought in the late 1960s to exist, now being discounted as a figment of incorrect callibration But satellite-borne detectors do measure the present day influx, whereas lunar microcrater counts measure the mean influx over the last few tens of thousands of years

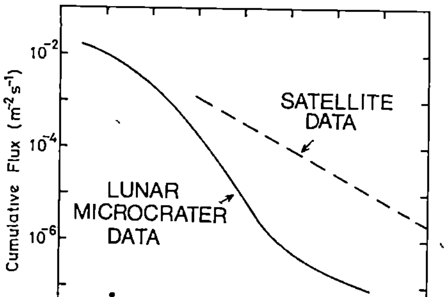
To convert the crater population on a given lunar sample into an interplanetary dust flux the size and form of the craters have to be related to the mass of the causative meteoroid One immediate problem is that crater size also depends on meteoroid velocity, dens ty, and shape Up to now the meteoroid impact velocity has been thought to be about 20 km s^{-1} Two recent results indicate that it is much lower Doppler shift observations of the solar spectral lines scattered by the solar system dust cloud give a geocentric velocity of about 5 km s^{-1} and this observation has recently been backed up by measurements of microcraters found in glass, steel and pyroxene surfaces exposed for 46 days during the Skylab mission Nagel, Fechtig, Schneider and Neukum (Max-Planck-Institut, Heidelberg) reported these observations at the recent COSPAR Meeting (Sao Paulo, 1974) They compared the spall zones, smoothness of the central depression, fracture damage and general shape of the 47 observed craters (diameters >1 μm) with the form of craters produced by simulated micrometeorites, these particles having been accelerated using a Van der Graff accelerator to known velocities in the 1 to 20 km s^{-1} range. In many cases these characteristics indicated the relatively low impact velocity of about 7 km s^{-1}. So both the curves in the figure have to be moved to the right by factors varying with the velocity dependence of microphone response, penetration hole size and crater diameter. As these effects are functions of meteoroid momentum or kinetic energy the use of this new velocity determination is still not sufficient to bring the curves together.

Two other things are assumed when dealing with microcraters, first that the ratio of the diameters of the microcrater pit and the impacting particle η is 2 and second that the density of the particle is 3 g cm^{-3}. Both these assumptions need to be reconsidered, recent laboratory experiments have shown that η decreases with particle density and also that the mean particle density varies with mass. Both these factors make the interpretation of microcraters more difficult. Measurements of crater circularity have led Brownlee (University of Washington) *et al* (4th Lunar Science Conference, Houston, 1973), to the conclusion that the majority of microparticles have equidimensional shapes and that shallow craters are produced by oblique incidence so the direction of the incident flux has also to be taken into account.

The main problem however lies in estimating the time the rocks under consideration have remained exposed on the lunar surface. One way of doing this is to count the tracks in the rock surface produced by retarding solar flare particles. Knowing the present day flare flux and assuming that solar activity has remained constant for about 10^6 years gives the exposure age. This second assumption is the stumbling block, the data of Neukum and Schneider could indicate that either a lower particle flux existed in the past or that the solar flare flux was higher then than now—both would produce the same result.

McDonnell, Ashworth, Flavill, Bateman and Jennison (University of Kent) overcome this problem by considering surfaces with an equilibrium distribution of craters. Laboratory simulation indicates that solar wind sputter erosion (0.043 Å yr^{-1}) is the dominant factor determining crater lifetime. Knowing this erosion rate enables the microparticle influx to be derived from the equilibrium distribution. The authors conclude (COSPAR, Sao Paulo, Brazil) that the microparticle influx has increased by a factor of four in the last 10^5 years.

This would account for the discrepancy shown in the figure but creates the question of why this flux has increased.



Number of micrometeoroids, with mass greater than the given mass, which will impact every second on a square metre of exposed surface one astronomical unit from the Sun

Adaptations of plants to drought

from Peter D Moore

THE C_4 dicarboxyl acid pathway of carbon fixation has now been recognised in a wide variety of plant species from at least six orders of flowering plants. In this process CO_2 combines with phosphoenolpyruvate and is transported to the bundle sheath cells in the form of dicarboxylic acids, where it is released as CO_2 to be fixed by the conventional Calvin (C_3) system. The widespread occurrence of the mechanism suggests that it arose independently in more than one order (Evans in *Photosynthesis and Photorespiration*, edit by Hatch, Osmond and Slatyer, 130, Wiley, New York, 1971). This being so, it has been difficult to study the evolutionary development of the process. Yet the work of Bjorkman *et al* (*Yb Carnegie Instn Wash*, **68**, 620, 1969 and **69**, 640, 1970) indicates that very few genes are involved.

Troughton (in *Photosynthesis and Photorespiration*, 124, 1971) has proposed that a study of the isotopic ratio of ^{13}C to ^{12}C (this is normally expressed in relation to a standard and is termed $\delta^{13}C$) in plant tissue should indicate the relative importance of the two systems of carbon fixation at the time when the plant was alive. This is because C_3 plants discriminate more effectively against ^{13}C in their carbon uptake than do C_4 plants, as a result C_3 plant tissue has an average $\delta^{13}C$ of -29‰ (that is proportionately less ^{13}C than in the air) whereas C_4 plants' average ^{13}C is -14‰.

Troughton, Wells and Mooney (*Science*, **185**, 610, 1974) have now used this technique to study the relative importance of the C_4 pathway over the past 40,000 years in the C_4 desert plant *Atriplex confertifolia*. Two ancient middens of the pack rat *Neotoma* from the Mohave Desert region of Nevada yielded material of this species which dated from >40,000 yr (beyond the scope of radiocarbon determination) and 10,000 yr respectively. The $\delta^{13}C$ of the older sample is -13.4‰ and that of the younger is -16.2‰. A modern sample of *A confertifolia* yielded a $\delta^{13}C$ of -14.8‰. Thus this species has been using the C_4 pathway for more than 40,000 yr.

Troughton *et al* also determined the $\delta^{13}C$ of *Opuntia polyacantha* tissues from the same sites. This species is a desert succulent which also concentrates carbon dioxide before fixing it by the conventional mechanism, but this time by crassulacean acid metabolism in which malic or citric acid concentrations build up during the night and the carbon accumulated in this way is subsequently used in the Calvin cycle. Once again these crassulacean acid (CAM) plants do not discriminate as strongly against ^{13}C as normal Calvin plants, and their $\delta^{13}C$ values resemble those of C_4 plants. In *Opuntia polyacantha* the >40,000-yr-old material gave a $\delta^{13}C$ value of -21.9‰, and the 10,000-yr-old sample gave -13.9‰. This suggests that the degree to which *Opuntia* depended upon the CAM technique of carbon concentration increased over the time interval between the two samples.

It is known that CAM plants become more dependent on night-time accumulation of carbon for their photosynthesis and growth under conditions of water stress. Indeed this mechanism can be regarded as an adaptation to dry habitat conditions in which normal daytime photosynthesis renders a plant liable to desiccation. The change in the $\delta^{13}C$ values of the *Opuntia* tissue analysed may, therefore, reflect the changing contemporary climatic conditions in Nevada. Other plant remains found in the area support this suggestion, since they give evidence of pluvial juniper woodlands, corresponding to the Wisconsin glaciation of the north, giving way to arid desert at about 10,000 yr b p.

There are similar indications from the *Atriplex* material. C_4 plants generally and *Atriplex confertifolia* in particular are frequently associated with rather dry habitats, their cellular resistance to CO_2 exchange is apparently lower than in C_3 plants, which may account indirectly for their capacity to withstand drought. It is significant, therefore, that the remains of *Atriplex* are considerably more abundant in the 10,000-yr-old specimen than in the 40,000-yr-old one. Thus palaeontological and palaeobiochemical evidence provide complementary information concerning the climatic changes during this period.

articles

Proterozoic crustal distribution, mobile belts and apparent polar movements

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The geological significance of a Precambrian supercontinent deduced from palaeomagnetic results is evaluated. A major transition in global tectonics took place during Upper Proterozoic and Lower Palaeozoic times. Older mobile belts developed along reactivated zones with slight movements of cratonic forelands but were related in time to apparent polar movements. The 1,150±200 Myr mobile belts formed along a single broad arc characterised by anorthositic and silicic intrusion over a long period of Proterozoic time.

PALAEOMAGNETIC studies of Precambrian and Palaeozoic rocks are yielding at present considerable quantitative information on the distribution and movements of the continental regions, and on their relationship to contemporary orogenic belts during these times. Formation of the supercontinent Pangaea involved the welding of several crustal units in Palaeozoic times[1] and the Caledonian orogenic belt developed along the line of closure of a restricted ocean basin[2]. These are two examples of the operation of plate tectonics during an interval for which seafloor spreading data are not available.

There is now substantial evidence that these examples are amongst the earliest expressions of this phenomenon of plate tectonics. The Upper Proterozoic to Lower Palaeozoic was characterised by a transition in global tectonics from a phase of localised internal deformation of large continental bodies to a phase of fracture, translation and collision of essentially rigid plates[3]. Palaeomagnetic results have demonstrated that Lower Palaeozoic and older mobile belts in Africa formed between essentially contiguous cratons[4]. A suggestion that Africa and North America have closely similar apparent polar wander (APW) paths before 1,000 Myr (refs 4 and 5) has been substantiated by detailed assessment of the data[6], and the paths can be closely superimposed over their whole lengths from 1,000 Myr to 2,200 Myr and possibly 2,700 Myr. When this is done the Afro-Arabian and North American regions form a continuous continental body (Fig 1). Palaeomagnetic results from Gondwanic continents other than Africa, although sparse, are consistent with them occupying their Pangean configuration with respect to Africa in Precambrian times[6]. Australia, however, seems to have changed its orientation sometime between 1,800 Myr and 750 Myr ago. This conclusion has been confirmed by a revised interpretation of the Late Precambrian–Lower Palaeozoic data[7]. Orientations of the Baltic–Ukrainian and Siberian Shields with respect to this reconstruction are uncertain but Donaldson *et al*[8] have recognised comparable trends in the North American and Baltic Shield APW curves and have superimposed them to place the two regions close to their present positions between 1,950 and 1,200 Myr.

Present interpretations of Precambrian palaeomagnetic results have two important implications. First they imply that orogenesis, and the behaviour of continental tectonism cannot be directly related to subduction at plate margins. Second, they show that the bulk of continental crust was lumped together as a single body through much of Precambrian time, although paucity of data from European and Siberian regions does not clarify whether or not they were continuously part of such a supercontinent. Here, I examine other evidence for these contentions and examine their implications.

Genesis of mobile belts

The finding that the major Precambrian mobile belts did not form by subduction at destructive plate margins prompts a search for alternative mechanisms. Granites are an important component of these belts and their upward migration through the crust as mobile bodies[9,10] could cause orogenic folding with zero crustal shortening[11]. This rise of relatively less dense material through a more dense sedimentary–volcanic cover is a mechanism amenable to

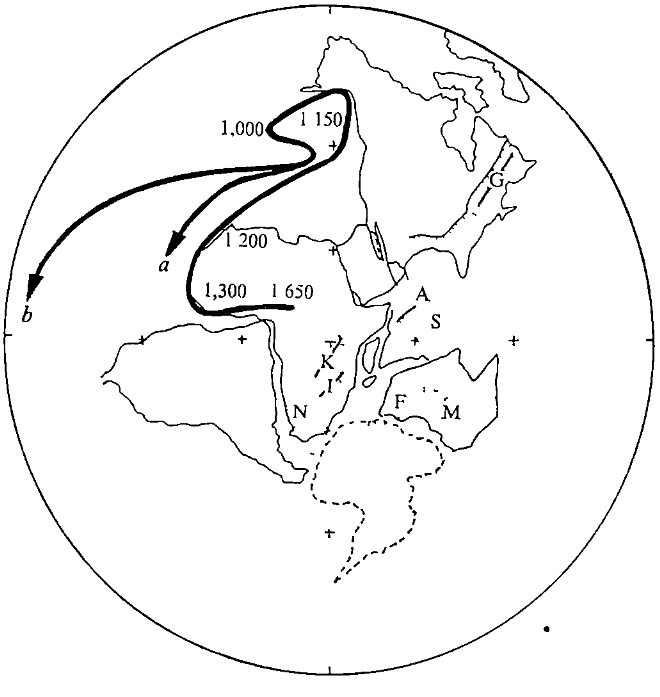

Fig 1 Stereographic reconstruction of the Gondwana continents and North America before about 1,000 Myr BP derived from palaeomagnetic data[6]. Orogenic belts belonging to the 1,150±200 Myr episodes N, Namaqualand, I, Irumide, K, Kibaran, A, Aravalli, S, Satpura, F, Frazer, M, Musgrave, G, Grenville. The apparent polar wander path spanning their formation is shown *a*, North America, *b*, Africa. Ages in Myr.

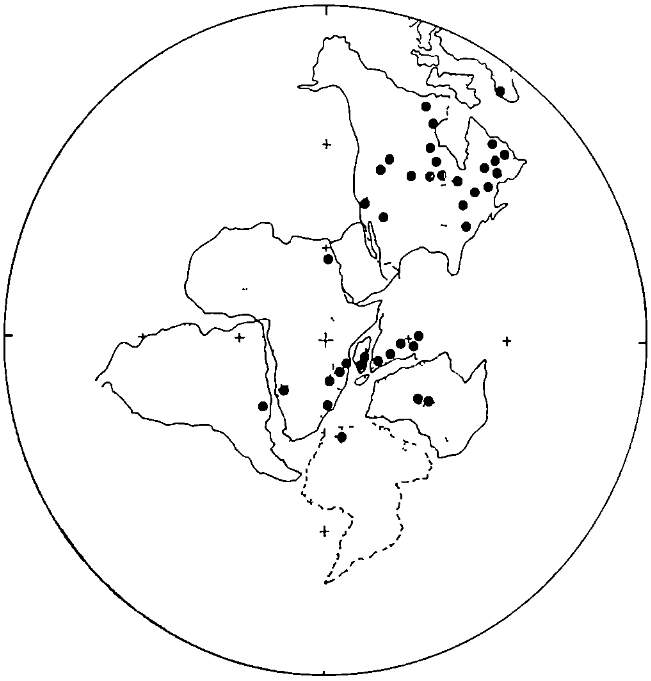

Fig 2 Distribution (stereographic projection) of Precambrian anorthosites (●, refs 37, 39) and granulites (stippled)[24,42] within the Proterozoic supercontinent The granulites in part represent relict rocks which have not been reconstituted by later events[19] and may thus indicate a paucity of fluids rather than the absence of any later thermal events

quantitative analysis, and Ramberg[12] has shown it to be a physically realistic process in mobile belts with a time span in the range 10–100 Myr Shackleton[13] has reached a comparable conclusion by regarding the granite magmas as, in part, a differentiate from rising mantle currents, and has argued[14] that progressive addition of granite magmas to the crust occurred during Precambrian time

This is an obvious mechanism but it is important to clarify the conditions which confine granite intrusion to the narrow zones of basement reactivation along which the Proterozoic belts formed[3 13 15] The absence of high pressure–low temperature metamorphic assemblages, and the widespread occurrence of granulites in Precambrian terrains have been rationalised by assuming a higher thermal gradient Two implications follow from this First, the crust may have been thinner and second, partial melting must have taken place at shallow depths, presumably leaving a granulate residuum The migration of fluids from the surrounding rocks may be necessary if significant upward movement of granites is to occur[16] and the crustal levels which they finally reach will then either reflect the point at which the magma chilled beyond its solidus, or the point at which further upward movement was prevented because sufficient fluid could not be abstracted[16 17] The concept of fluids is important base metal mineralisation is practically confined to the Proterozoic mobile belts in Africa, with the cratons barren except for the gold–silver associations of the greenstone belts[18] Paucity of fluids may also explain why large areas of Archaean granulites were not amphibolitised in later times[19] It is unlikely that Proterozoic belts have experienced much differential erosion with respect to the Archaean cratons, because platform sediments on the cratons can be correlated with those in the mobile belts[20] and their depositional history suggests that the cratons underwent intermittent uplift during Proterozoic times[21] A possible explanation is that the cratons were added to by underplating with andesitic or granitic material[14] whereas the appropriate fluids concentrated to permit upward migration of granite diapirs only in zones later defined by the orogenic belts and metallogenic provinces

Given that mobile zones define accessions of heat and/or fluids at the base of the lithosphere, their distribution must be related in some way to properties of the Proterozoic asthenosphere The distribution of mobile belts and related features on the Proterozoic continental reconstruction is consistent with such a conclusion

Precambrian continental distribution

The Proterozoic continental reconstruction (Fig 1) is applicable from at least 2,200 Myr to shortly after 1,000 Myr BP It is not possible to match the segments of the APW curves for North America and the Gondwana continents[6] between approximately 950 and 650 Myr ago Australia seems to have changed its orientation during Proterozoic times[22–24] to its Phanerozoic configuration[25,26] Before 1,000 Myr sialic crust was distributed as a broad girdle across about 140°, or 15,500 km, of arc and with the inferred placing of the Baltic Shield[8] this is extended to about 190° or 21,000 km The agreement of palaeomagnetic results with a single APW curve across this supercontinent[6] demonstrates that it was disposed on a globe of comparable radius to the present The reconstruction is based on palaeomagnetic data, with uncertainties which accommodate the limited intercontinental movements necessary to explain the intrusion and deformation of basic dyke swarms, the infolding of sediments and the small relative motions of forelands of mobile belts Indeed, it is entirely compatible with internal deformation and metamorphism taking place within a coherent entity, as proposed by Sutton and Watson[3] Whether or not this is to be regarded as a crustal plate[11] in the modern context is academic

The belts of the 1,150±200 Myr mobility, now isolated by later drift, were an essentially unified feature lying on a broad arc close to a great circle spanning the Proterozoic supercontinent These belts had a protracted history In Africa the Kibaran belt experienced major tectonism about 1,300 Myr BP with post orogenic granites intruded approximately 1,250 Myr ago and with the subparallel Irumide belt undergoing synorogenic events about 1,100 Myr ago The

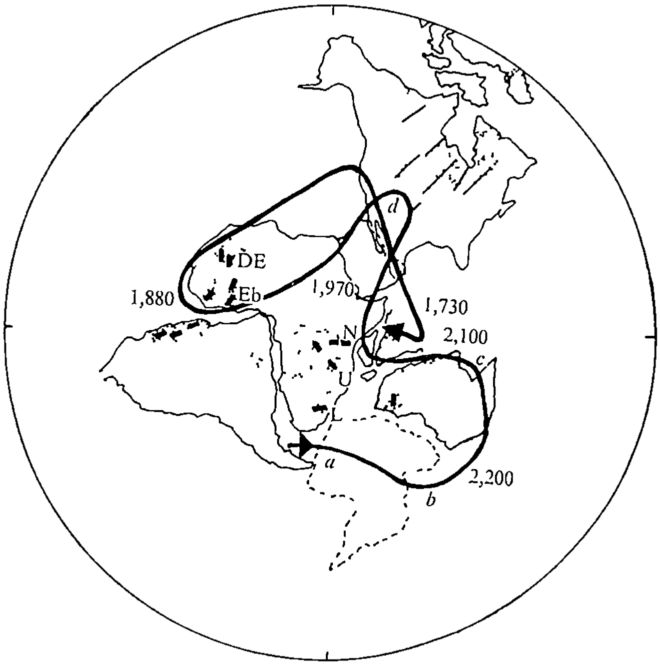

Fig 3 The relationship of recognisable pre-1,150±200 Myr orogenic belts with cratonic regions[24,40], and the apparent polar wander paths executed during their formation The belts are L, Limpopo, N, Nyanzian, U, Ubendian, Eb, Eburnean, DE, Dorsal Eglabs The ages of the belts are not precisely defined but the youngest (DE) was folded about 2,100 Myr ago The straight lines in North America and Greenland are straight belts of late Archaean–early Proterozoic age[19] The generalised APW curve is a composite one which reconciles differences between published APW curves for North America[8 44] by incorporating a 2,000–1,700 Myr loop identified from African data[4] *a–d*, See text, dotted areas, crust unaffected by thermal/tectonic events after about 2,000 Myr BP

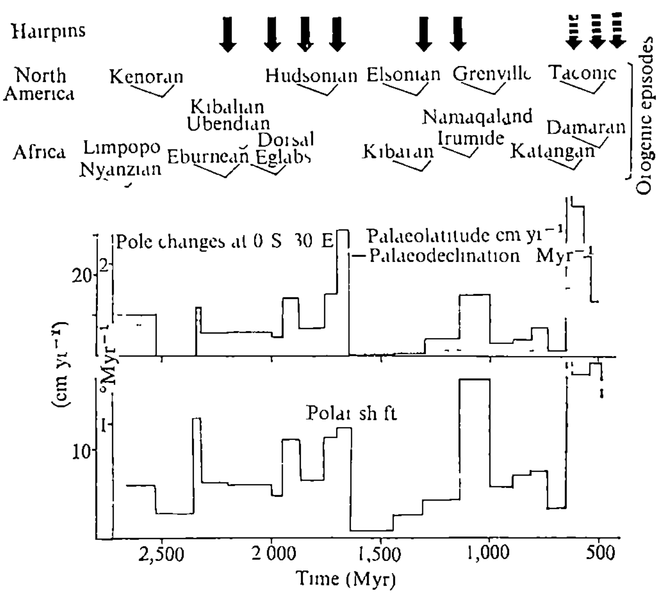

Fig 4 North American and African orogenic episodes shown in relation to hairpin bends (dashed arrows, the Gondwanic continents) and the rate of apparent polar wandering The lower histogram shows rates of angular change in the palaeomagnetic pole and the palaeolatitude of a point in central Africa at 30°E, 0°S Where both of these are large it is probable that the APW reflects crustal drift

Namaqualand (Orange River) belt of south-western Africa, which experienced a widespread thermal event, with remobilisation of a granite gneiss basement at about the same time, seems to be a linear continuation of these belts, although it has been isolated by the development of the pan-African belts (750–450 Myr) The 1,400–1,050 Myr Frazer–Musgrave belt of Australia[27], which, before Phanerozoic drift, connected with rocks of comparable age in the Davis Sea region of Antarctica[24], is brought into parallelism with belts of a similar age elsewhere using the Carey–Hurley Rand reconstruction The Musgrave is regarded by Rutland[28] as a narrow zone of basement reactivation with a high geothermal gradient, and it underwent a main phase of metamorphism 1,330 Myr ago

The continuity of the 1,150±200 Myr orogenic belts is emphasised by identification of rocks about 1,000 Myr old within the pan-African domain of north-eastern Africa (ref 29 and A M Chater personal communication), by the presence of 1,100 Myr old anorogenic rocks in the Arabian Shield[30], and by the parallelism of the contemporary Aravalli belt of north-western India (ref 21 and Fig 1) Stockwell[31] and Hurley and Rand[24] have distinguished a wide zone affected by a thermal (?) episode about 1,300 Myr ago in the North American Shield, bordering the Grenville Belt, and extensive anorogenic igneous activity of similar age[32] has been recognised in southern Greenland, north of the extrapolated continuation of the Greenville belt In common with the later pan-African episode, restricted zones of linear orogenesis were apparently bordered by wider zones of reheating and igneous intrusion

If the reconstruction of Donaldson *et al*[8] is correct then the 1,150±200 Myr mobile belt may have been even longer than indicated in Fig 1 Those authors place the Baltic Shield with the Gothide Belt on a great circle continuation of the Grenville Belt Evidence for a sialic connection is however, lacking There is substanial palaeomagnetic evidence[33,34] that the Grenville Province became attached to the remainder of the North American Shield by predominantly dextral strike-slip plate motion about 1,150 Myr ago and there has been a suggestion[8] that a similar motion may apply to the juncture of the Gothide Belt with the Baltic Shield Alternative interpretations are possible, however, and palaeomagnetic data[4] are not consistent with comparable movement along the contemporaneous African belts Along this whole belt there is close spatial relationship between the orogenic belts, postorogenic intrusion and subsequent sedimentation During and immediately before tectonism the belts were subject to extensive graben faulting and deposition of clastic rocks Keweenawan sediments formed in tensional rifts along the Grenville Front[35] as did Gardar sediments in Greenland and Jotnian sediments in Scandinavia[36] Extensive and lithologically similar Upper Proterozoic sequences which outcrop across central Africa include the Bukoban and Kundelungu Groups, developed in a comparable setting to the Kibaran, Irumide and Namaqualand belts[20,21,29]

Unlike the later supercontinent Pangaea, which was essentially a transient feature[1] evolved and dismembered by plate tectonic mechanisms, the Proterozoic supercontinent (Fig 1) existed for a very long period of time (at least 1,200 and possibly 1,700 Myr), and geological events before the 1,150±200 Myr orogenies may be expected to show a simple distribution on the reconstruction This is indeed the case, and the two broad NE–SW belts of granulites and Archaean cratons which have been recognised on the Pangean reconstruction[24] are brought on to a single line (Fig 2) Also, regions of massive Proterozoic anorthosites, previously regarded as two separate belts ranging from western North America to the Urals and South America to India[37], are in fact seen to be one continuous broad belt These massive anorthosites (Fig 2) contrast with the anorthosites intimately involved with Archaean granite gneisses[38] and their intrusion possibly spanned an interval from 2,200 Myr to 1,000 Myr BP They represent one facet of magmatism[36] that also includes a Rapakivi granite phase, occurring along what is now seen to be a single zone of high thermal activity and block faulting which developed after 2,200–1,750 Myr mobile episodes

The massive anorthosites are widely regarded as having an upper mantle origin, and it is believed that they required both high temperatures and pressures for their formation[39] According to Buddington[39] they were intruded during an

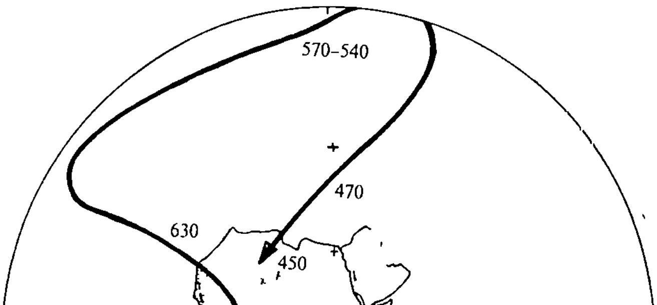

Fig 5 The pan-African belts (750–450 Myr), showing generalised structural trends and the APW path executed during the interval of their formation Based on the interpretation of McElhinny *et al*[8] The Gondwanaland reconstruction is based on refs 25 and 26, a more southerly position for Madagascar as proposed by Tarling[43] would place all continental units to the right of East Africa approximately 10° further south For simplicity present continental outlines are used in these figures although they include crust accreted in later times

interval of high temperature gradients such as have not been attained in later times It is notable that their intrusion ceased at the same time (about 1,000 Myr) as large differential movements of discrete plates first occurred

The broad belt of magmatism and later tectonic activity is not clearly recognisable as a crustal lineament earlier than about 1,700 Myr, and older Proterozoic belts (>1,750 Myr) have a distribution apparently unrelated to it The older belts cannot be followed as linear features for distances of more than 100 km Although that could be a consequence of overprinting by later episodes, where the belt are widely exposed they lie along small circles (Fig 3 and refs 13, 20 and 40), in contrast to the arc close to a great circle of the 1,150±200 Myr belts Correlation of Proterozoic mobile belts with lines of upwelling[13] or downgoing[40] mantle convection currents could explain this in terms of a change in the convective systems

Mobile belts and apparent polar wandering

The parallelism of the Kibaran–Grenville trend and the large contemporary polar shift is a striking feature of Fig 1 The relationship implies that the continental body executed a large lateral movement along an arc close to a great circle during the interval of deformation and metamorphism That this and a later apparent polar shift (Fig 5) represent real translation of the continent, rather than pure polar wandering, is suggested by considerable palaeoclimatic evidence[6,7] But it is not possible to equate directly rates of crustal drift and polar wandering, because these will only be equal when the Euler pole is equidistant from the source and palaeomagnetic pole[1] A minimum estimate of crustal drift can be obtained, however, from the change in palaeomagnetic latitude and the amount of local rotation inferred from changes in declination I have computed these two parameters for a point 30°E, 0°S in central Africa to show that high crustal rates of drift coincide with both the 1,150±200 Myr and the pan-African episodes, with low drift rates characterising the quiescent period between the >1,750 Myr and 1,150±200 Myr episodes (Fig 4) The large lateral shifts are terminated by 'hairpins'[8] in the APW curve, which seem to correlate closely with tectonic episodes This relationship is particularly clear for the 1,150±200 Myr events In these cases the 1,300–1,200 Myr hairpin corresponds to the climax of the Kibaran–Elsonian episode, and the 1,150 Myr hairpin corresponds to tectonic events in the Namaqualand, Irumide and Grenville Belts In addition, two principal events of the later pan-African episode, the Katangan (680–580 Myr) and Damaran (550–500 Myr) (ref 38) are both broadly coincident with hairpins (Fig 5) and the major structural trends of this episode in eastern and western Africa They could be correlated with the major polar shifts which occurred between 650 and 500 Myr ago although this has yet to be clarified

Are hairpins and large polar shifts related to earlier orogenic episodes? In most cases age control does not provide adequate information to permit any decision about which part of the APW curve corresponds to particular episodes There is one possible exception in the southern part of the West African craton, and in comparable parts of the Guyana craton, the Eburnean tectonism took place along ENE lines about 2,200 Myr ago Pre- and syntectonic palaeomagnetic poles from the region lie on segment *a–b* of the APW curve (Fig 3) whereas post-tectonic poles lie on section *b–c* with hairpin *b* occurring synchronously with the tectonic event Further north in West Africa, in the Dorsal Eglabs, north–south tectonic trends at least 1,970 Myr old are sub-parallel to a segment of the curve defined by syn- and post-tectonic poles from the region (Fig 3, *c* and *d*) These correlations go a long way towards establishing a relationship between Proterozoic linear mobile belts and mantle processes Given that they are produced by zones of asthenosphere which are hotter, and/or contain more volatiles, than surrounding regions, migration of the lithosphere with respect to a mantle source, as evidenced by the polar shifts, would be capable of producing linear belts Such mantle sources may be comparable to the thermal highs to which recent magmatism is widely related (for example ref 4)

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Model for the regulation of mRNA translation applied to haemoglobin synthesis

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A kinetic analysis of protein synthesis on ribosomes predicts that certain treatments which reduce the overall rate of polypeptide chain initiation will inhibit translation preferentially of mRNAs with lower rate constants for polypeptide chain initiation The effects of several inhibitors on α and β-globin synthesis by reticulocyte extracts agree with these predictions

SEVERAL factors determine the rate of initiation of translation of an mRNA molecule First, different mRNAs seem to differ in the rate of attachment to ribosomes and/or in other stages of initiation of protein synthesis For example, in the reticulocyte each molecule of α-globin mRNA initiates protein synthesis only 60% as frequently as does each β-globin mRNA[1,2] Similarly, the frequencies of initiation of translation of the ten monogenic reovirus mRNAs differ at least tenfold[3] and the rates of initiation of translation of the three cistrons of bacteriophage RNA differ more than twentyfold[4-8] In this latter case, it has been proposed that the primary nucleotide sequence about the initiation codon and/or the tertiary structure of the mRNA near this codon determine the rate of ribosome attachment[5,7]

Second, under certain conditions there are alterations in the overall rate of polypeptide chain initiation in a cell For instance, during mitosis the rate of chain initiation is only one third of that during other phases of the cell cycle, although the rate of polypeptide chain elongation is unaltered[9] In reticulocytes, the absence of haemin results in formation of an inhibitor of binding of the initiator Met-tRNA$_f$ to 40S ribosomes[10-12], initiation of synthesis of all reticulocyte proteins is restricted[13-15]

In this communication I derive a simple kinetic rate equation for initiation and elongation of polypeptide chains in eukaryotic cells A principal result of these calculations is that any reduction in the rates of polypeptide chain initiation steps at or before binding of mRNA will result in preferential inhibition of translation of mRNAs with lower initiation rate constants (the poorer mRNAs) I present several new results on translation of α and β-globin mRNA in the rabbit reticulocyte which are predicted by, and consistent with, this conclusion I will show, further, that changes in the levels or rate-limiting components required for initiation of synthesis of all proteins (for example, initiation factors, Met-tRNA$_f$, 40S subunits, and so on) could explain many results previously ascribed to mRNA specific factors

The model

Polypeptide synthesis on ribosomes can be described by the following scheme

$$m + R^* \xrightarrow{K_1} P_1 \xrightarrow{K_2} P_2 \xrightarrow{K_3} P_3 \xrightarrow{K_4} \qquad \xrightarrow{K_S} P_S \xrightarrow{K_R} P_R$$

The binding of Met-tRNA$_f$ to the 40S subunit is the first step in protein synthesis in eukaryotic cells[16-19], R^* is the concentration of the Met-tRNA$_f$-40S ribosome complex able to bind an mRNA, m is the concentration of the mRNA, and K_1 is the rate constant for ribosome binding and formation of the 80S initiation complex P_1 Although there are several factors and several steps involved in this process, the use of an overall rate constant K_1, possibly different for different mRNAs, is sufficient and does not affect the nature of the final rate equation It is assumed that mRNA-specific factors are not involved in mRNA binding The polypeptide contains S amino acids and the message S codons, P_i (i=1, 2, , S) represents a nascent chain of i amino acids P_R is the released polypeptide chain K_i (i=2, 3, , S) is the rate constant for addition of the i th amino acid, and K_R is the rate constant for release of the completed polypeptide chain Ribosomes are known to cover many more codons on a message than those being decoded at any instant, the value L is the number of codons covered by one ribosome

MacDonald and Gibbs[20,21] have derived rate equations for polypeptide synthesis in a very general context, and my derivation follows closely their analysis It is necessary to make several simplifying assumptions in order to yield a relatively simple analytical expression which can readily be compared with experimental data First, we must assume that rate constant for addition of any amino acid is the same, that is $K_i(i=2 \quad s)=K_e$ the single elongation rate constant Following ref 20 I define n_i as the probability that an mRNA contains a ribosome with a nascent chain i amino acids long I make the crucial assumption here that under all conditions ribosomes are uniformly distributed along an mRNA (see below), that is that $n_i = n$ for all $i=1, \quad , s$ It is also assumed that $K_R \leqslant K_e$ that is that release of the completed chain does not limit the overall rate of protein synthesis Given these assumptions, the flux of ribosomes, q_e across any given codon in an mRNA is given by equation (10) of ref 20

$$q_e = K_e n (1 - nL) [1 - n(L - 1)]^{-1} \qquad (1)$$

In the steady state this value represents the net amount of protein synthesised per mRNA, hence the total amount of protein produced per unit time, Q_e, is given by

$$Q_e = mq_e = mK_e n (1 - nL) [1 - n(L - 1)]^{-1} \qquad (2)$$

The number of new polypeptide chains initiated per unit time, Q_i is given by the following equation

$$Q_i = mR^* K_1 (1 - nL) \qquad (3)$$

where the term in brackets is the probability that the initiation codon is not covered by a ribosome and, hence, available for polypeptide chain initiation This equation follows from the definitions of K_1 and R^* (ref 21)

Since, in the steady state, the number of new chains initiated is balanced by the number elongated or completed, we can set $Q_i = Q_e$ and solve for n

$$n = [L - 1 + K_e(K_1R^*)^{-1}]^{-1} \qquad (4)$$

and combining equations (3) and (4), $Q = Q_i = Q_e$ is given by

$$Q = mR^*K_1 \left[1 - \frac{L}{\frac{K_e}{K_1R^*} + L - 1}\right] \qquad (5)$$

The average number of ribosomes per mRNA is equal to nS, and the fraction of codons on a message covered by a ribosome is given by nL In comparing the translation of two different types of mRNA in the same cell, I assume that K_e (and R*) are the same for all mRNAs In the case of greatest interest here, the rabbit reticulocyte, we have snown directly that the rate of elongation of the α and β polypeptides is the same[2] K_1 (and hence n) will, in general, be different for different mRNAs

R*, the concentration of Met-tRNA$_f$-40S complex capable of binding mRNA, is a key element in this equation, it can be taken as a measure of the overall rate of polypeptide chain initiation in the cell Reductions in the rate of formation of 40S subunits, or of binding of Met-tRNA$_f$ to them, will result in a lowered value of R*, likewise, addition of an inhibitor of mRNA binding, such as poly(I) on aurintricarboxylic acid, will also result in a reduction of R*

The crucial assumption in the derivation of equation (5) is that ribosomes are on the average distributed uniformly along an mRNA, that is $n_i = n$ for all i This is the case for the α and β-globin mRNA in the normal rabbit reticulocyte[22,23] In the specific experimental situations considered below—inhibition of polypeptide chain initiation in the reticulocyte—it can safely be assumed that in the steady state ribosomes will continue to be distributed uniformly on the average (but at a lower density) However, in cases where initiation is increased from the normal, relative to chain elongation (high values of R*) the assumption of uniform distribution may no longer be valid These cases have been considered theoretically by MacDonald *et al*[20,21]

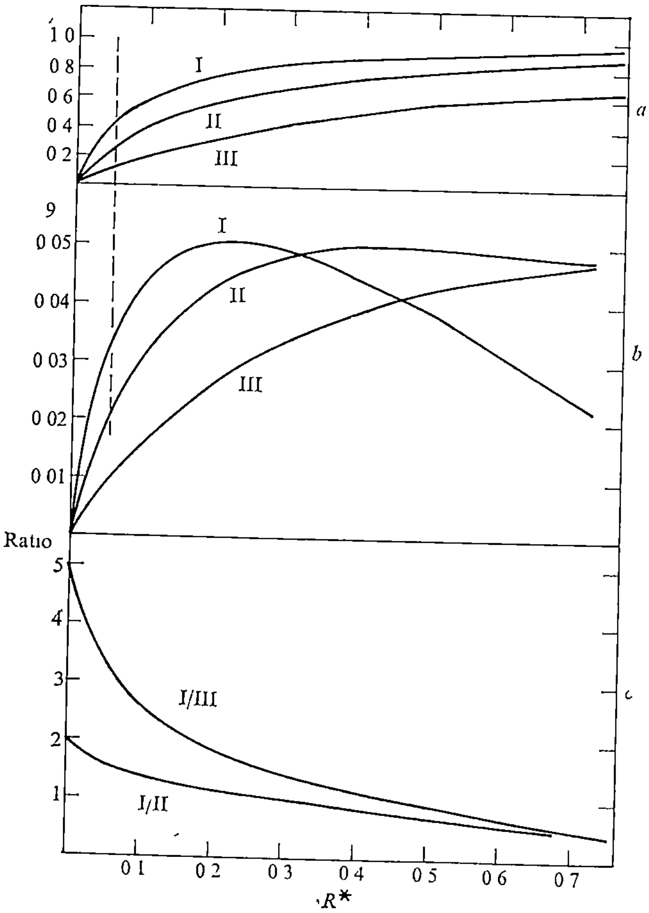

Fig. 1 Theoretical calculation of parameters of protein synthesis as a function of R* A value of 1 is assumed for K_e (rate constant for elongation) and 12 is taken for L, the number of codons covered by one ribosome Rate constants for chain initiation, K_1, are taken as 1 0 (curve I), 0 48 (curve II) and 0 20 (curve III) *a*, The fraction of codons occupied by a ribosome, nL, plotted as a function of R*, using equation (4) *b*, Amount of protein synthesised per mRNA, $q = Qm^{-1}$ plotted as a function of R*, using equation (5) *c*, Ratio of amount of protein synthesised by the different mRNAs, plotted as a function of R* As detailed in the text translation of β and α-globin mRNAs can be approximated by curves I and II, respectively The dashed line represents the conditions obtaining in the normal reticulocyte, where n^{β} (curve I) = 0 0340 and n^{α} (curve II) = 0 0211

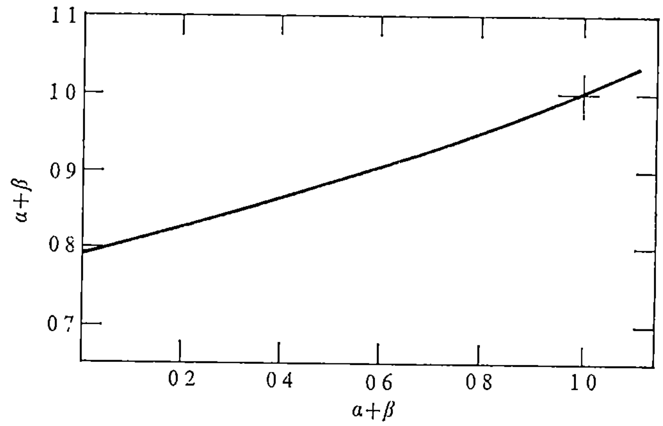

Fig 2 Theoretical calculation of the effect on relative synthesis of α and β-globin chains by inhibiting polypeptide chain initiation a stages at or before binding of mRNA The values, $K^{\alpha}_1 - K^{\beta}_1 = 0\ 48$ and $m^{\alpha} - m^{\beta} = 1\ 57$ were calculated, as detailed in the text, using only the result that, under normal conditions obtaining in the reticulocyte, each α mRNA contains, on the average, three ribosomes while each β contains five These values and equation (5) were used to calculate the ratio of α to β chains produced as a function of total globin synthesis, for the latter a value of 1 0 corresponds to synthesis in the uninhibited reticulocyte

In Fig 1*b* the relative translation of three mRNAs is plotted as a function of R* with values for K_1 set at 1 (curve I), 0 48 (curve II) and 0 2 (curve III) The value of K_e is set arbitrarily at 1, and a value of 12 for L is used Since a molecule of β-globin mRNA (147 codons) can apparently contain a maximum of 12 ribosomes[24] a value of 12 (=144−12) appears reasonable, it is consistent with the size of mRNA protected from nuclease digestion by a ribosome[8] (The relative positions of the three curves in this panel is unaffected if $L=7$ or $L=20$ is used)

Note that, with increasing R*, the rate of protein synthesis increases to a maximum, and then declines This means that above a certain density of ribosomes on the mRNA ribosomes interfere significantly with movement of each other along the mRNA (The optimum value of n can be calculated from equation (2) by setting $dq/dn=0$, it is given by equation (7) of ref 21) It should be stressed that, because of the assumption $n_i = n$ used in deriving equation (5), the curves in Fig 1 may not be correct at high values of R*

The crucial point to emerge from Fig 1 is that the relative translation of two mRNAs with different values of K_1 changes monotonically with R* (Fig 1*c*) At very low values of R* the amount of protein synthesised by a species of mRNA is given by

$$Q_{lim} = mK_1R^* \qquad (6)$$

Increasing the value of R* results in proportionally greater stimulation of translation of the mRNA with a lower value of K_1 Conversely, reducing R* (inhibiting chain initiation nonspecifically) results in a proportionally greater inhibition of translation of the mRNA with lower K_1

Application to synthesis of α and ß globin

Cell-free extracts of rabbit reticulocytes synthesise globin for about 1 h at rates comparable to that of the intact cell These extracts, as do the intact cells, synthesise equal amounts of α and β-globin chains[1,21] Each message for α globin contains on the average five ribosomes ($n^{\beta} = 5/147 = 0\ 0340$) while each α-globin mRNA contains three ($n^{\alpha} = 3/142 = 0\ 0211$)[1,26] Since the rate of elongation of the two chains is the same[2], I conclude that under conditions obtaining in the cell each α mRNA

Table 1 Inhibition of synthesis of α and β globins

Reaction	Time of labelling	% Total globin synthesis	Ratio α β globins synthesised
Control (plus haemin)	0–10	100	1 02
Minus haemin	0–10	94	0 99
Plus double-stranded RNA 10^{-3} µg ml^{-1}	0–10	92	0 98
Plus double-stranded RNA 3×10^{-3} µg ml^{-1}	0–10	93	0 96
Plus preincubated lysate	0–10	18	0 83
Control (plus haemin)	10–30	100	1 02
Minus haemin	10–30	17	0 80
Plus double-stranded RNA 10^{-3} µg ml^{-1}	10–30	27	0 86
Plus double-stranded RNA 3×10^{-3} µg ml^{-1}	10–30	15	0 75
Control (plus haemin)	0–20	100	1 01
Plus ATA 2×10^{-5}M	0–20	45	0 91
4×10^{-5}M	0–20	21	0 82
Plus poly(dI) 6 6 µg ml^{-1}	0–20	63	0 92
13 2 µg ml^{-1}	0–20	17	0 75
Plus poly(dT) 6 6 µg ml^{-1}	0–20	61	0 89
11 4 µg ml^{-1}	0–20	38	0 80
Plus pactamycin 5×10^{-8} M	0–20	75	1 11
1×10^{-7} M	0–20	54	1 13
2×10^{-7} M	0–20	17	1 29

Reticulocyte cell-free protein synthesis reactions (0 06 ml) have been described in detail[2,53] They contained 2×10^{-6}M nonradioactive tyrosine, and ^{14}C-tyrosine (5 µCi ml^{-1}, 480 mCi mmol^{-1}, New England Nuclear Corp) was added during the indicated labelling period Unless otherwise noted, reactions also contained 20 µg ml^{-1} haemin Aliquots of the reaction were taken to measure total incorporation of radioactive tyrosine into protein After incubation, about 600,000 c p m ^{3}H-tyrosine-labelled rabbit haemoglobin was added Globin was isolated and digested with trypsin, the tyrosine-containing peptides were resolved by paper electrophoresis and detected by autoradiography (ref 2) The measure of α-chain synthesis was the average of the ratios of ^{14}C to ^{3}H radioactivity in peptides αT4 and αT6, β-chain synthesis was the ratio of ^{14}C to ^{3}H radioactivity in peptide βT4 These three relatively N-terminal peptides were utilised so that peptide labelling represents predominantly (over 98% in the control reaction) polypeptide chains initiated during the course of the cell-free reaction

initiates protein synthesis only 60% as frequently as does each β mRNA and that there must be 1 7 (=1 –0 6) times as much α mRNA as β mRNA[1,2] Further studies showed that any putative α-mRNA-specific or β-mRNA-specific initiation factors or specific ribosomes do not limit the rate of α or β-globin synthesis (unpublished results of G Temple and myself)

Since at the value of R* which obtains in the riboculocyte $n^{\beta}-n^{\alpha}=1\ 6$, I calculate from equation (4) that $K_1{}^{\alpha}=K_1{}^{\beta}=0\ 48$ Since $Q^{\alpha}=Q^{\beta}$, from equation (3) I calculate that $m^{\alpha}-m^{\beta}=1\ 57$, in agreement with a previous estimate (ref 1) The probability that an mRNA will have no ribosomes translating it is given by $(1-n)^{s}$ For α mRNA in the reticulocyte lysate ($n^{\alpha}=0\ 0211$, $s=142$), this value is 0 049 while for β mRNA ($n^{\beta}=0\ 0340$, $s=147$) it is 0 0062 Recent evidence showed that there is indeed 10 times more α mRNA unattached to ribosomes than β mRNA[27 28]

The relative translation of α and β mRNAs, as a function of R*, are thus given by curves II and I, respectively, of Fig 1*b* In Fig 2 are plotted the same data but in a form which eliminates R* — the ratio α β as a function of the total rate of polypeptide chain initiation (the sum of α and β chains synthesised) Figures 1*b* and 2 make one crucial prediction any treatment which (nonspecifically) inhibits the rate of polypeptide chain initiation at stages at or before mRNA binding will preferentially inhibit translation of α-globin mRNA

Table 1 shows the results of experiments involving six different treatments, all of which do not affect polypeptide chain elongation or termination, and all of which block initiation at steps at or before binding of mRNA All preferentially inhibit translation of α mRNA The data in this table follows very closely the theoretical curve of Fig 2

When a reticulocyte lysate is incubated in the absence of haemin, protein synthesis is normal for about 8 min (at 30° C) Synthesis continues at a decreasing rate, and ceases by 15–30 min, concomitantly there is buildup of an inhibitor which blocks binding of Met-tRNA$_f$ to the 40S subunit[10-15] Table 1 shows that there is equal synthesis of α and β globin during the first 10 min in reactions with or without haemin, and also from 10 to 30 min in the presence of haemin By contrast, in a lysate incubated in the absence of haemin there is a marked reduction in the ratio α β synthesised from 10 to 30 min, during the time inhibition is manifest In a second experiment, a lysate was incubated for 1 h in the absence of haemin, to build up the initiation inhibitor, and then a small amount was added to a fresh lysate Again, the amount of α chain made in the second reaction was only 70% that of β (Table 1)

Globin synthesis in the presence of 10^{-2} µg ml^{-1} double-stranded RNA (in the presence of haemin) is similar to that of a reaction in the absence of haemin[13,29,30] There is also produced in this case an inhibitor which blocks binding of Met-tRNA$_f$ to the 40S subunit[31] Table 1 shows that double-stranded RNA has no effect on the amount of α or β chains made during the first 10 min of incubation By contrast, during the next 20 min, when inhibition of initiation is manifest, there is preferential inhibition of synthesis of α globin

Aurintricarboxylic acid, at low concentrations, is a specific inhibitor of chain initiation[32,33] It blocks the binding of mRNA to the Met-tRNA$_f$-40S complex (ref 34 and unpublished data) In kinetic terms ATA can be viewed as lowering the concentration (R*) of Met-tRNA$_f$-40S complexes which can bind mRNA Table 1 also shows that ATA preferentially inhibits initiation of synthesis of α chains

A large number of synthetic polyribonucleotides and polydeoxyribonucleotides are also specific inhibitors of chain initiation and also block binding of mRNA to the Met-tRNA$_f$-40S complex (ref 34 and unpublished data) Like ATA, these can be viewed as reducing the concentration of R* We have shown that all 11 polynucleotides tested preferentially block α-chain synthesis[35] Table 1 shows recent results for two of those, poly(dT) and poly(dI)

It is interesting that the initiation inhibitor pactamycin which acts at a stage after binding of mRNA to the Met-tRNA$_f$-40S complex[36,37] does not preferentially inhibit α-chain synthesis This result is not in disagreement with the theory, which only pertains to steps at or before mRNA binding These results give confidence that my theory is correct at least as pertains to synthesis in rabbit reticulocyte lysates

Application to other systems

That one can alter in a predictable manner the relative translation of different mRNAs by nonspecific changes in polypeptide chain initiation components has implications for other systems In cases where the rate of cellular protein synthesis is either increasing rapidly with time—such as after fertilisation of the quiescent sea urchin embryo (reviewed in ref 38)—or decreasing—such as after infection of cultured cells by poliovirus[39]—one might reasonably expect that different mRNAs would be affected to different extents

Although the above calculations assume that mRNA-specific factors do not regulate polypeptide chain initiation, they could easily accommodate them, in this case K_1 for any mRNA would be a function of the concentration of such factors Whether mRNA specific factors do, in fact, exist is at present controversial In this context it is useful to point out the three eukaryotic systems available which can translate exogenous mRNA at very high efficiency (greater than 10 molecules of protein produced per mRNA added) The rabbit reticulocyte lysate[40,41], the mixed cell-free system of Schrier *et al*[42] and the *Xenopus* oocyte (into which the mRNA is microinjected)[43] In each of these systems a wide variety of exogenous mRNAs have been translated and in no case is there any evidence for mRNA specificity Palmiter has shown for instance, that a molecule of ovalbumin mRNA is translated in a crude reticulocyte lysate with nearly the same rate of chain initiation and elongation as obtains in the intact hen oviduct[41]

In contrast, RNA-specific factors have been observed in cell-free systems, such as preincubated extracts from mouse ascites cells[44-50], in which there is synthesis of, at best, one molecule of protein per molecule of mRNA added[49] It is clear that in such extracts some component or components required for polypeptide chain initiation (and elongation) are limiting (ref 51 and reviewed in ref 52) The present theory shows that addition to such extracts of a component required for initiation for all mRNAs can stimulate preferentially translation of certain mRNAs It is at present difficult to prove or disprove the notion that mRNA-specific factors, are, in fact, nonspecific components which are required for translation of all mRNAs, particularly since the K_1 constant of a mRNA could be changed considerably by reagents used in its isolation, such as organic solvents and detergents Addition of unfractionated rabbit globin mRNA to an extract of ascites cells generally results in synthesis of about twice as much β globin as α globin Nudel *et al*[44] reported a factor preparation from reticulocyte ribosomes which will preferentially stimulate translation of α mRNA in the ascites extract At least in the intact reticulocyte α mRNA has a lower K_1 than β mRNA, the present theory predicts that addition of whatever initiation factor limits synthesis in the ascites extracts would preferentially stimulate α-globin synthesis Whether this factor, and the others[45-49] supposedly specific for translation of β-globin mRNA or myosin mRNA are real entities, artefacts of cell-free synthesis, or, as postulated here, nonspecific components required for all chain initiation, remains for future work

Finally, the theory developed here is applicable to transcription of DNA by RNA polymerase, under conditions of limitation of some factor required for transcription initiation, the theory predicts a selective inhibition of transcription of those operons with a lower rate constant for polymerase attachment and RNA synthesis initiation.

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Gene deletion as the cause of *α* thalassaemia

Two independent groups show that the absence of all or part of the globin α-chain gene is the origin of the homozygous α thalassaemia

The severe form of α thalassaemia is caused by a haemoglobin gene deletion

THE thalassaemias are a group of common genetic disorders characterised by a reduced rate of synthesis of either the α or β chains of human adult haemoglobin (Hb A, $\alpha_2\beta_2$), resulting in associated clinical disorders[1,2,3] α Thalassaemia results from a deficiency of α chains, which causes an apparent excess in production of γ chains of foetal haemoglobin (Hb F, $\alpha_2\gamma_2$) in foetal life and an excess of β chains of haemoglobin A in adult life The excess γ and β chains produce the abnormal tetramer haemoglobins Bart's (γ_4) and H (β_4) respectively Although the genetics of α thalassaemia are still not fully understood, three important clinical varieties of α thalassaemia result from the interactions of three different mutations in allelic genes α thalassaemia 1 (α-thal 1), α thalassaemia 2 (α-thal 2), and haemoglobin Constant Spring (CS)[1,4,5] The homozygous state for the α-thal 1 mutation (Hb Bart's hydrops fetalis syndrome) results in intrauterine or early neonatal death with a clinical picture of hydrops fetalis and a haemoglobin pattern consisting entirely of Hbs Bart's, H, and Portland ($\zeta_2\gamma_2$), with complete absence of α-globin chain synthesis[6] The doubly heterozygous states for either α-thal 1 and α-thal 2 mutations, or α-thal 1 and CS mutations, result in Hb-H disease, a moderately severe haemolytic anaemia consistent with survival into adult life[1]

There is evidence that in some populations (including those in south-east Asia), the human α-chain locus is duplicated and there are at least two α-chain loci per chromosome[1] The Hb Bart's hydrops syndrome might result from inactivity of all the α loci whereas in Hb-H disease might be loss of function at one out of the four[7-14] A deletion of all or part of the sequence of the α-globin genes could prevent the synthesis of functional mRNA Alternatively, the α-thalassaemia mutations might affect the control of transcription or translation in such a way as to result in a complete inactivity of α-chain gene The site of mutation could then be remote from the α-globin structural genes

We have tried to distinguish these different models by a study of the hybridisation of the DNA of an α-thal 1 homozygote to purified probes prepared from globin messenger RNAs with viral reverse transcriptase We conclude that the α-thal 1 mutation is a gene deletion affecting at least a substantial proportion of all of the α-globin cistrons

Human globin messenger RNA was prepared[15,16] from adult reticulocyte-rich blood from a non-thalassaemic haemolytic anaemia ($\text{mRNA}_{\alpha,\beta}{}^{r}$), umbilical-cord blood from newborn infants obtained at exchange transfusion ($\text{mRNA}_{\alpha,\gamma,\beta}{}^{r}$), normal foetal liver ($\text{mRNA}_{\alpha\ \gamma\ \beta}{}^{f}$), and foetal liver from an infant homozygous for α-thal 1 The infant died 20 min after delivery, haemoglobin electrophoresis showed only Hb Bart's and the minor components typical of the Hb Bart's hydrops syndrome[6]

Both normal and thalassaemic foetal livers were highly erythropoietic The mRNAs were usually isolated by affinity chromatography using poly(U)—sepharose[17,18] from total polysomal RNA prepared under conditions minimising RNA breakdown[19] Wherever possible the translational capacity of the mRNAs was tested in a heterologous protein synthesis system, either by microinjection into *Xenopus* oocytes[20] or using a cell-free system from Krebs ascites tumour cells[21] or wheat germ[22] The samples were also examined by polyacrylamide gel electrophoresis where possible to evaluate the purity of the mRNAs, but sometimes the small amounts obtained precluded such an analysis

A complementary DNA copy (cDNA) was prepared to various human globin mRNAs with avian myeloblastosis virus reverse transcriptase[23] using labelled ^{3}H-dCTP only cDNA was centrifuged on an alkaline sucrose gradient and the material larger than approximately 5S (300 nucleotides) was pooled and used for further hybridisation cDNAs were prepared using mRNA from adult ($\text{cDNA}_{\alpha\ \beta}{}^{r}$) or newborn ($\text{cDNA}_{\alpha,\gamma,\beta}{}^{r}$) reticulocytes as templates, in these cases, unlike that of foetal liver, kinetic analysis demonstrates that only very low amounts of cDNAs are homologous to mRNAs for proteins other than globin[24] The newborn reticulocytes synthesise approximately equal amounts of haemoglobins A and F

Hybridisations of $\text{cDNA}_{\alpha,\beta}{}^{r}$ and $\text{cDNA}_{\alpha\ \gamma,\beta}{}^{r}$ to their template mRNAs show that each cDNA preparation contains approximately 5–10% labelled material which behaved as double stranded even in the absence of hybridisation, and a further 10–15% which did not hybridise to its template even at very high mRNA/cDNA ratios, and which may consist of very short oligonucleotides None of the data presented here have been normalised (the hybridisation values have not been corrected to allow for the apparently double-stranded or the apparently non-hybridisable cDNA and to equate the observed hybridisation to 100%) since this would obscure the extent of errors which might arise in the experiments Both $\text{cDNA}_{\alpha,\beta}{}^{r}$ (from adult reticulocyte mRNA) and $\text{cDNA}_{\alpha,\gamma,\beta}{}^{r}$ (from newborn exchange-transfusion reticulocyte mRNA) hybridised back to their templates to the extent expected[24]

The same result was obtained when $\text{cDNA}_{\alpha,\beta}{}^{r}$ or $\text{cDNA}_{\alpha,\gamma,\beta}{}^{r}$ are hybridised to total mRNA prepared from normal human foetal liver (Fig 1) But when hybridisation was attempted to total mRNA isolated from liver homozygous for α-thal 1 homozygote, the result was different, with each cDNA hybridising at normal mRNA/cDNA ratios only to approximately 55% of that possible Since the complexity of these cDNAs is approximately 800 nucleotides (data not shown, see ref 24), indicating that most of the cDNA is complementary to only two to three mRNAs of the size of globin mRNA, we can interpret the hybridisation observed as due to only β (or β and γ) cDNA components Any major contamination with cDNAs derived from other mRNAs would have been detected in the kinetic analysis of the hybridisation of cDNA to templates We conclude that the $\text{cDNA}_{\alpha}{}^{r}$ did not hybridise due to the absence of mRNA for α globin from the α-thal 1 liver This is consistent with the complete absence of α-globin chain synthesis in the thalassaemic foetal material[6]

Nuclear DNA was prepared from normal human spleen and from liver and spleen of the Hb Bart's hydrops infant The DNA was purified on hydroxylapatite and sonicated in alkali to an average molecular weight of 100,000 (ref 25)

The sonicated normal and thalassaemic human DNA were then mixed individually with globin $\text{cDNA}_{\alpha,\gamma,\beta}{}^{r}$ and allowed to reanneal (Fig 2) The reannealing of the total nuclear DNA was followed optically by measuring directly the proportion of the A_{260} bound (reannealed) and unbound (single stranded) to hydroxylapatite Under our conditions about 20% reanneals rapidly, indicating its presence in many copies This value is slightly lower than previously published[26], a discrepancy explicable by the differences in size of DNA fragments and method of hybrid analysis The remainder of the reannealing curve represents sequences present in one or only a few copies[27]

The hybridisation of the human foetal globin $\text{cDNA}_{\alpha,\gamma\ \beta}{}^{r}$ to excess normal human DNA, as measured by binding to hydroxylapatite, demonstrates that the genes for α, γ and β globin all reanneal with the kinetics of the non-repeated portion of the genome The hybridisation to normal DNA gives a $C_0t_{1/2}$ of approximately 700, slightly more rapid than the $C_0t_{1/2}$ of the total DNA which is 1,000 This might be predicted from

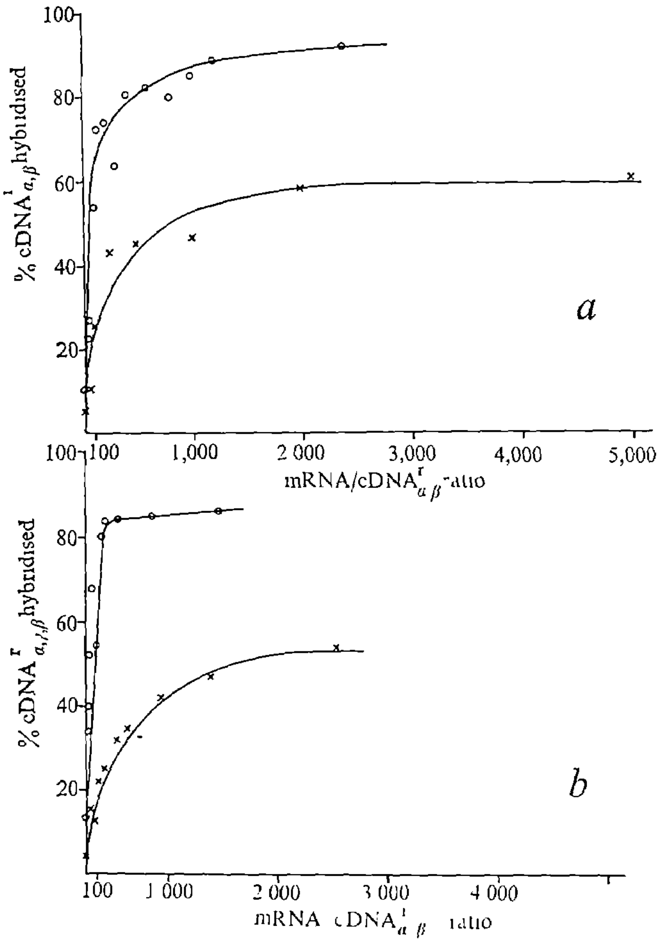

Fig 1 Hybridisation of liver mRNA from normal and α thalassaemic foetuses to cDNA$_{\alpha,\beta}^r$ and cDNA$_{\alpha,\gamma,\beta}^r$ Human globin mRNA was prepared from ribosome pellets of reticulocyte lysates from adult non-thalassaemic anaemic patients (mRNA$_{\alpha,\beta}^r$) and from newborn infants exchange transfused due to Rh incompatibility (mRNA$_{\alpha,\gamma,\beta}^r$) The pellets were extracted by a phenol–chloroform method[33], and the RNA precipitated by the addition of two volumes of ethanol The precipitate was dissolved in NETS buffer (100 mM NaCl, 1 mM EDTA, 10 mM Tris, pH 7 4, 0 2% SLS) and poly(A) containing mRNA purified by a cycle of poly(U)-sepharose chromatography[18] Each mRNA was tested in the wheat germ cell-free system[22] for biological activity mRNA from adult reticulocytes directed the synthesis of 57% α and 43% β-globin chain, mRNA from foetal reticulocytes of 55% α, 32% γ and 13% β-globin chain Counts under globin peaks accounted for at least 80% of the total radioactivity incorporated over the background endogenous synthesis A single-stranded DNA copy (cDNA) of each mRNA was prepared as described previously[23,30], using AMV reverse transcriptase, and with ^{3}H-dCTP (13 3 Ci mmol^{-1} Amersham) as the only labelled deoxynucleotide, upon centrifugation in alkaline sucrose gradients, both cDNAs, prepared with adult or foetal mRNA had a peak molecular weight of approximately 1 1 × 10^5, material larger than 0 9 × 10^5 was pooled and used for subsequent experiments Thalassaemic foetal liver was obtained from an infant with the Hb Bart's hydrops fetalis syndrome immediately after death Normal foetal liver was obtained from *post-mortem* material within minutes of death Livers were homogenised in 3–5 volumes of a solution containing 50 mM NaCl, 1 5 mM MgCl$_2$, 100 mM Tris, pH 7 6, 100 mM sucrose, 0 1% diethylpyrocarbonate, and nuclei were removed by centrifuging twice at 2,000g for 10 min The supernatant was diluted with 5–10 volumes of NETS buffer, and directly extracted with phenol–chloroform RNA was ethanol precipitated and mRNA was isolated by poly(U)-sepharose chromatography 120 pg (specific activity 7 5 × 10^6 c p m μg^{-1} cDNA calculated assuming dCTP to be 25% of the incorporated deoxynucleotide) of each cDNA were incubated in sealed sterile silicone-treated capillaries with increasing amounts of their template RNAs in 4 μl of a hybridisation solution containing 0 5 M NaCl, 25 mM Hepes, 10 mM EDTA pH 6 8, 50% formamide, 500 μg ml^{-1} *Escherichia coli* RNA[23] After 6 d at 43° C, the percentage of cDNA which had hybridised was determined by assay with the single-strand specific nuclease S$_1$ followed by perchloric acid precipitation of the undigested material The indicated RNA/cDNA ratio has been determined from the amount of added 9S RNA (as calculated by polyacrylamide gel electrophoresis analysis of poly (U)-bound RNA) The material which bound to the poly(U)-sepharose column (poly(A)-containing RNA) was hybridised to cDNA$_{\alpha,\beta}^r$ (*a*) and cDNA$_{\alpha,\gamma,\beta}^r$ (*b*) The unbound material did not contain any appreciable amount of globin sequences as determined by hybridisation to cDNA The indicated amount of RNA refers to the total poly (U)-bound material Hybridisation of *a*, cDNA$_{\alpha,\beta}^r$ and *b*, cDNA$_{\alpha,\gamma,\beta}^r$ to normal (○) and α-thal 1 (×) foetal liver mRNA

the fact that there are at least two genes for γ globin and probably two genes for α globin[1] Seventy-five per cent of the cDNA hybridises at a DNA cDNA ratio of 2 × 10^7 The hybridisation of cDNA$_{\alpha,\gamma,\beta}^r$ to the thalassaemic DNA gives a $C_0t_{1/2}$ of approximately 400 but the final amount of cDNA hybridising is only 60% a deficit of approximately one-quarter compared with normal DNA

This non-hybridisation of a portion of the cDNA$_{\alpha,\gamma,\beta}^r$ to the thalassaemia DNA suggests that the genes for α-globin have been deleted To confirm this cDNA enriched in sequences complementary to α-globin mRNA was prepared by two methods In the first, it was assumed that the foetal liver mRNA from the Hb Bart's hydrops fetalis infant contains no mRNA$_\alpha$ cDNA$_{\alpha,\beta}^r$ from adult reticulocytes was hybridised to the mRNA prepared from liver of the Hb Bart's hydrops fetalis infant at an mRNA/cDNA ratio sufficient to give 50% hybridisation of the cDNA The hybridised sequences (putative cDNA$_\beta^r$) were then bound to hydroxylapatite and the non-hybridised cDNA$_\alpha^r$ (α-enriched cDNA) which did not bind was collected At the usual RNA/cDNA ratios the cDNA$_\alpha$ hybridises to α thal 1 liver mRNA to a small extent, approximately 20%, as compared to mRNA$_{\alpha,\beta}^r$ or normal foetal liver mRNA, for which hybridisation is to 70% At very high ratios of approximately 3,000 1 mRNA/cDNA somewhat greater hybridisation (to approximately 30%) occurs between the cDNA$_\alpha$ and the mRNA from the α-thal 1 homozygote We estimate the α-enriched cDNA contains approximately 75% cDNA$_\alpha^r$

The second method for preparing α-enriched cDNA used mRNA prepared from reticulocytes from a patient with homozygous β-thalassaemia as a template for reverse transcriptase From the ratio of cell-free α β+γ chain synthesis the cDNA was estimated to be 80–90% cDNA$_\alpha^r$

The reannealing experiments at vast DNA excess with normal and α thalassaemic DNA were repeated using cDNA$_\alpha^r$ as a probe (Fig 3) The two cDNA$_\alpha^r$ probes gave indistinguishable annealing profiles with normal and thalassaemic DNAs The total DNA reanneals with a $C_0t_{1/2}$ of approximately 1,000 as in the previous experiments and the cDNA$_\alpha^r$ reanneals with the normal human DNA to give a curve very similar to that for cDNA$_{\alpha,\gamma,\beta}^r$ with a $C_0t_{1/2}$ of 700 The cDNA hybridises to 70% In the case of the homozygous α-thal 1 DNA, a quite different picture is seen Hybridisation occurs only very slowly and a final value of 40% cDNA$_\alpha$ hybridised is obtained at a C_0t of greater than 3×10^4, thirty times greater than the $C_0t_{1/2}$ of non-reiterated DNA sequences, as estimated from the ratios of the C_0ts of the low hybridisation values measured

The results obtained with normal globin cDNA$_{\alpha,\gamma,\beta}^r$ and cDNA$_{\alpha,\beta}^r$ demonstrate that, as in the case of duck[28,29] and mouse[30], human globin genes reanneal with the non-reiterated component of the DNA, or slightly faster, and are present as only single or a few copies This is consistent with the current genetic evidence indicating 2–4 human γ and α globin loci[1]

The very different rate of hybridisation for the normal and α-thalassaemic DNAs with both purified cDNA$_\alpha^r$ and cDNA$_{\alpha,\gamma,\beta}^r$ cannot be explained by a single nucleotide mutation which affects transcription or translation The extent of hybridisation of the thalassaemic DNA never exceeds 30% of that of the normal DNA, even at very high C_0ts, demonstrating the deletion of all, or at least a substantial part, of the genes for α-globin mRNA Since there are almost certainly two α-chain genes in this population, this suggests that they are closely linked The cDNA$_\alpha$ hybridisation to 70% with normal total DNA demonstrates that α-globin genes are present in large excess relative to cDNA in these experiments

A deletion of more than half of the α-globin gene at all loci would give the annealing profiles seen in Figs 2 and 3 As the cDNA is transcribed from the poly(A) sequence on the 3′-end of the mRNA, it is particularly rich in sequences at the 3′-end of the gene, coding for C-terminal sequences of the protein, and therefore the extent of the deletion will be underestimated Moreover, the purified cDNA$_\alpha{}^r$ is still contaminated with approximately 25% sequences complementary to other globin mRNAs

A component of the reannealing seen with the purified cDNA$_\alpha{}^r$ and α-thal DNA might be due to mismatched hybridisation to the genes for the α-type chain zeta (ζ). This early embryonic chain forms tetramers with γ chain in haemoglobin Portland ($\zeta_2\gamma_2$) in early embryonic life, and is known to be present in α-thal 1 homozygotes at approximately 1–5% of the total globin[6] Preliminary studies indicate that it contains some α-chain-like sequences, particularly at the C-terminal end[32] Furthermore, it behaves functionally as an α chain, since the tetramer $\zeta_2\varepsilon_2$ (the probable formula for haemoglobin Gower 1[33]) shows complete cooperative function The slow rise in hybridisation between cDNA$_\alpha{}^r$ and mRNA from α-thal foetal liver at very high mRNA/cDNA ratios may also be due to mismatched mRNA–cDNAα hybrid formation.

We conclude, therefore, that a substantial, and in all probability complete, deletion of the genes for α globin is the molecular mechanism which produces the α-thal 1 disorder and hence, in its homozygous state, the Hb Bart's hydrops syndrome Extrapolating from our results with α-thal 1, it seems likely that in many cases α-thal 2 results from the loss of a single α-gene locus Since there is now strong evidence that the heterozygous state for Hb CS also mimics the clinical picture of α-thal 2, it is possible that some cases of α-thal 2 result from a molecular mechanism other than a deletion

From a practical point of view, it is perhaps discouraging that a disorder such as this severe form of α thalassaemia, which causes a major public health problem in many parts of the world, results from a gene deletion, since this precludes any possibility of correction at the post-transcriptional level

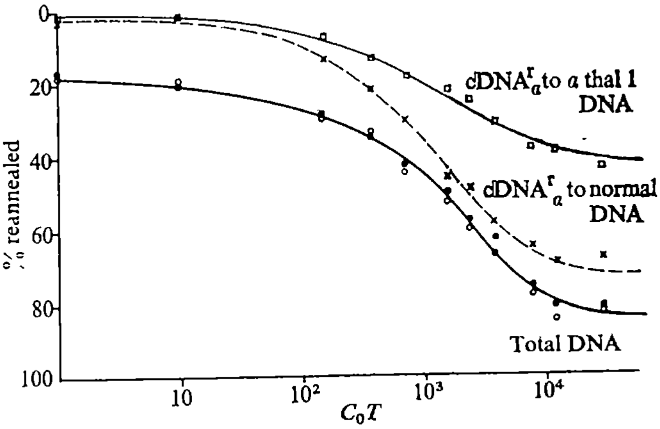

Fig 3 Reassociation of α-enriched cDNA to excess normal or α-thalassaemic total DNA α-Enriched cDNA (0 4 ng) prepared by hydroxylapatite adsorption was mixed with excess (25 × 10^6 l^{-1}) normal or α-thalassaemic DNA at a concentration of 5 mg ml^{-1} in phosphate buffer, and hybridised as in Fig 2 Percentage of radioactivity in single-stranded DNA × (normal DNA) and □ (α-thalassaemic DNA) Percentage of absorbance in single-stranded DNA ○ (normal DNA) and ●, (α-thalassaemic DNA) In a control experiment α-enriched cDNA was hybridised with a 20 × 10^6 excess of *E coli* DNA 3% of the cDNA$_\alpha$ was eluted with the double-stranded fraction at C_0t0, 8% at C_0t 4,500, 9% at C_0t 10,000 The slight increase of cDNA eluted in the 0 4 M phosphate fraction at very high C_0t after hybridisation with *E coli* DNA has been previously reported[23]

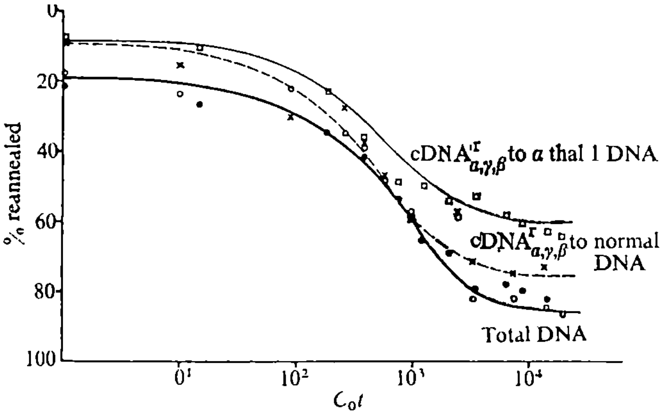

Fig. 2 Reassociation of cDNA$_{\alpha\ \gamma,\beta}{}^r$ to excess normal or α-thalassaemic total DNA DNA was prepared from nuclei of normal human spleen or of hydrops fetalis liver with hydroxylapatite[25], and sonicated in 0 1 NaOH to an average size of approximately 300 nucleotides DNA fragments were neutralised, ethanol precipitated, redissolved in distilled water and dialysed three times against 20 volumes of water cDNA$_{\alpha\ \gamma,\beta}{}^r$ (0 5 ng) was mixed with excess (20 × 10^6 l^{-1}) normal or α-thalassaemic DNA at a concentration of 8 3 mg DNA ml^{-1} in 0 12 M sodium phosphate, *p*H 6 8 120 µl aliquots of the solution were sealed in sterile silicone-treated capillaries, the mixtures were denatured for 10 min at 100° C, then annealed at 60° C At the appropriate times, samples were removed and fractionated on hydroxylapatite columns[30] Reannealing of total DNA was followed by measuring spectrophotometrically the proportion of DNA eluted as single-stranded material (at 0 16 M sodium phosphate, *p*H 6 8) and double-stranded material (at 0 4 M sodium phosphate) Radioactivity of the 0 16 M and 0 4 M phosphate fractions was determined by counting directly in Instagel (Packard) In all cases, at least 95% of the radioactivity, or of the total DNA, was recovered as single-stranded or reannealed DNA by hydroxylapatite The percentage of radioactivity in single-stranded DNA is shown by × (normal DNA) and □ (α-thalassaemic DNA), that of absorbance in single-stranded DNA by ○ (normal DNA) and (α-thalassaemic DNA)

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Genetic lesion in homozygous α thalassaemia (hydrops fetalis)

HOMOZYGOUS α thalassaemia (hydrops fetalis) is invariably fatal to afflicted infants within 30–40 weeks *in utero* or soon after birth[1] In this form of α thalassaemia both the α chain of haemoglobin and the α-globin mRNA are undetectable[2,3] To define the nature of this defect we have examined the DNA from two cases of hydrops to determine whether major alterations in the structural gene for α globin are present

For these studies we prepared complementary DNA (cDNA) to total globin mRNA (α+β) from a non-thalassaemic patient with autoimmune haemolytic anaemia, as well as cDNAs specific for either α or β-globin mRNA sequences The procedures are detailed in the legend to Fig 1 Evidence for the specificity of the cDNAs was obtained by measuring the rates of annealing (expressed as $C_r t$ (mol s^{-1} l^{-1})) to an excess of globin mRNA The two cDNAs annealed at the same rate to globin mRNA from a non-thalassaemic individual (Fig 1*a*) When globin mRNA from an individual with haemoglobin H disease was used, the presumptive α-globin cDNA annealed at a rate about ten times slower than that of the β-globin cDNA (Fig 1*b*) In this disease there is a reduction of α-globin chain relative to production of β-globin chain and a corresponding reduction in the α-globin mRNA[9,10] When an aliquot of the mRNA used in the above experiment was translated with the cell-free system from the Krebs II mouse ascites tumour[5], the ratio of α to β globin synthesised was 1 9 Thus the different rates of annealing observed in Fig 1*b* are consistent with the presumption that the cDNAs used are specific for α and β-globin sequences

We then used these ^{32}P-labelled cDNAs to detect α and β-globin nucleotide sequences in DNA extracted from the livers of two Chinese infants with homozygous α thalassaemia and, as a control, a stillborn infant without thalassaemia Annealing of the ^{32}P-labelled cDNA during the reassociation of the unlabelled cellular DNA was monitored by hydroxylapatite fractionation A large excess of cell DNA was used to ensure that even sequences represented a single time in the human genome would anneal with the cDNA Our results are summarised in Fig 2, with experimental details provided in the legend In each experiment we in-

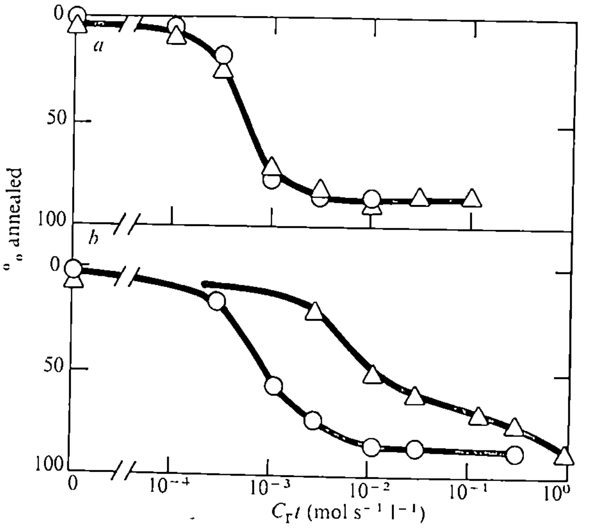

Fig 1 Annealing of globin cDNA to globin mRNA Reticulocytes were obtained from non-thalassaemic individuals or from patients with haemoglobin H disease RNA was extracted with phenol and sodium dodecyl sulphate[4], and analysed by rate zonal sedimentation RNA migrating in the range 7–11S was pooled and used without further purification These RNAs were translated using the cell-free system from Krebs II mouse ascites tumour[5] and the product was analysed by chromatography on CM-cellulose[6] The product translated from non-thalassaemic RNA contained equal amounts of α and β globin and from haemoglobin H RNA the ratio of α to β globin synthesised was 1 9 Both RNAs were transcribed into cDNA using RNA-directed DNA polymerase purified from Prague C strain of Rous sarcoma virus The conditions of synthesis were as follows 0 1 U ml^{-1} polymerase[7], 0 4 μg ml^{-1} RNA template, 0 1 μg ml^{-1} oligo $(dT)_{12-18}$ (Collaborative Research), 100 μg ml^{-1} actinomycin (Calbiochem), 0 1 M Tris (*p*H 8 1), 0 008 M $MgCl_2$, 0 02 M β-mercaptoethanol, 80 μM dATP, 80 μM TTP, 80 μM dGTP, 0 8 μM (α-^{32}P) dCTP (115 Ci $mmol^{-1}$, New England Nuclear Corp) Synthesis was for 2 h at 37° C using a reaction volume of 3 ml After the addition of 50 μg of yeast RNA, the nucleic acids were extracted with sodium dodecyl sulphate and pronase, followed by a single phenol extraction at room temperature The cDNA was precipitated with ethanol and treated with alkali (0 6N NaOH, 37° C, 1 h) to remove RNA The solution was neutralised and again precipitated with ethanol The cDNA was then annealed to an excess of its template (normal or haemoglobin H mRNA) under the following conditions 0 3 M NaCl, 0 001 M EDTA, 0 02 M Tris (*p*H 7 4), at 68° C That cDNA which annealed to RNA was isolated by batch elution from hydroxyapatite[8] and then passed through a column of Sephadex G-50 (40×0 9 cm) The excluded volume was collected, treated with alkali as described above and re-precipitated with ethanol That cDNA transcribed from RNA of the individual with haemoglobin H disease was used as a probe for β-globin sequences without further purification The cDNA transcribed from the normal RNA was subjected to further fractionation in an attempt to obtain probe specific for α-globin sequences First, the unfractionated cDNA was annealed to a limited extent with haemoglobin H RNA After incubation, that cDNA which had not annealed (30%) was isolated by hydroxyapatite fractionation and Sephadex G-50 chromatography, and subsequently treated with alkali and precipitated with ethanol as described above This adsorption process was repeated three more times in an attempt to remove sequences complementary to β-globin mRNA The remaining unadsorbed cDNA was finally annealed to an excess of normal RNA The annealed cDNA was recovered The experiment depicted in the figure shows evidence that this cDNA is specific for α-globin sequences The presumptive α-globin (△) and β-globin (○) probes were examined for their ability to anneal to an excess of RNA either from a normal individual (*a*), or from a patient with haemoglobin H disease (*b*) Hybridisation was assayed by batch elution from hydroxylapatite and results are plotted against the product of RNA concentration and time ($C_r t$)

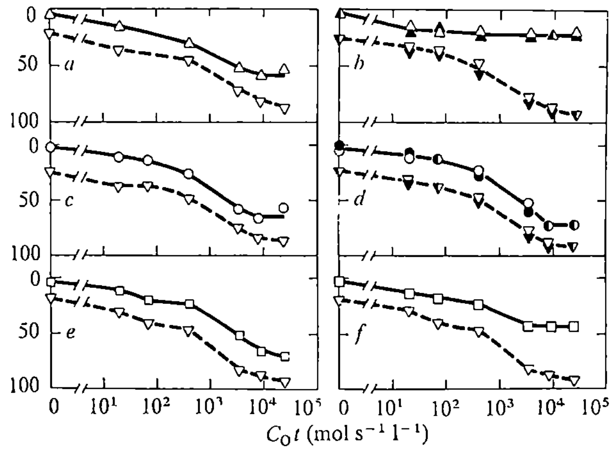

Fig 2 Annealing of labelled globin cDNA to cellular DNA Nucleic acid was extracted from the livers of two patients with hydrops fetalis using sodium dodecyl sulphate and pronase followed by two room temperature phenol extractions After treatment with ribonuclease and further phenol extractions, the DNA was reduced in size to about 2×10^6 daltons either by shearing at 50,000 pounds per square inch[12] or limited depurination[13] (0 3N NaOH, 100° C for 20 min) HeLa cells in suspension culture were labelled with ^{3}H-thymidine for 48 h and the DNA was isolated as described above Single-stranded ^{32}P-cDNA was prepared as described for Fig 1, and annealed under the following conditions 5 mg ml^{-1} cell DNA, 2400 c p m ml^{-1} ^{32}P-globin cDNA, 24,000 c p m ml^{-1} ^{3}H-HeLa DNA, 0 5 M NaCl, 0 002 M EDTA, 0 04 M Tris (*p*H 7 4), at 68° C Incubations were performed in 80 µl reaction volumes, under mineral oil, for from 20 min to 120 h Annealing was assayed using hydroxylapatite[14] and plotted against C_0t values corrected to standard conditions[15] *a* and *b*, α-globin cDNA (△, ▲) was annealed either with normal or hydrops DNA respectively Similarly (*c*) and (*d*) refer to β-globin cDNA (○, ●) annealed with normal or hydrops DNA Unfractionated globin cDNA (□) transcribed from normal mRNA was also annealed to normal (*e*) and hydrops DNA (*f*) In each panel of the figure the ^{3}H-labelled DNA (▽, ▼) acts as an internal control on the observed rate of annealing *b* and *d*, The DNA from two cases of hydrops was tested separately and the results are shown by the open and closed symbols

cluded a small amount of ^{3}H-labelled human DNA from HeLa cells and its annealing was followed to determine the rate of reassociation of the cellular DNA Figs 2*a* and *c* show that α and β-globin cDNAs annealed at about the same rate to DNA from the non-thalassaemic foetus In contrast, α-globin cDNA did not anneal significantly to DNA from the hydrops foetuses (Fig 2*b*) although β-globin cDNA reacted normally (Fig 2*d*) A similar result was obtained when unfractionated globin cDNA (α+β) was used The extent of annealing with non-thalassaemic DNA (Fig 2*e*) was about twice that observed with hydrops DNA (Fig 2*f*) We interpret that data to mean that at least part of the α-globin gene is absent from the DNA in hydrops, the amount of the β-globin genes appears to be normal

This conclusion is subject to several qualifications First, although the cDNA used is specific for α globin, it is not a uniform representation of the structural gene The average size of the cDNA used in the experiment was only 380 nucleotides, when measured by sedimentation in alkaline sucrose The complete α-chain mRNA is composed of about 630 nucleotides[16], about 60 of these are contained in the 3′-terminal poly(A) segment[17], adjacent to which is a segment of at least 93 nucleotides which are not normally translated[18] Since the cDNA used in our experiments was presumably transcribed from the 3′-end of the mRNA, it is likely to represent only a part of the sequence of the α-globin structural gene

Second, the observed rates of annealing of the cDNA to cell DNA depend on the relative excess of sequences in the cell DNA that are complementary to the cDNA The mass ratio of cell DNA/cDNA used was $6\ 7\times10^7$ If we assume the human haploid genome contains 3×10^9 nucleotide pairs and the average length of the cDNA is 380 nucleotides, then the ratio of unlabelled to labelled sequences is 8 5 1 This also assumes that the α-globin gene is represented only one per haploid genome Actually there is evidence that the gene might be duplicated[19] so that our sequence excess might be twice the value calculated above To confirm that these experiments were performed with adequate quantities of cell DNA we have performed annealings with as much as a fivefold greater excess of cell DNA relative to cDNA and have seen no more than a 10% increase in the extent of annealing We therefore deduce from the annealing rates in Fig 2*b* compared with those in Fig 2*d*, that the hydrops DNA contains less than 2% of the α-chain sequences detected in normal DNA Assuming that the DNA of each somatic cell is identical, then part or all of the α-chain gene is actually deleted In addition, if the α-chain gene is normally duplicated then in the hydrops both loci must be affected

In summary, we have shown that for homozygous α thalassaemia in Chinese subjects, the cellular DNA lacks certain sequences normally found in α-globin mRNA

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letters to nature

Highly polarised radio outburst from Cygnus X-3

We report here the results of extensive observations of a new series of outbursts from Cyg X-3 which we commenced following the recent IAU telegram alert from Sir Martin Ryle A high degree of linear polarisation has been discovered in one of the recent outbursts The degree of polarisation changes over a period of 17 d and reaches a maximum of 14% The observations indicate a high degree of order in the source magnetic field and that a thermal plasma coexists with the radiating relativistic electrons In addition, a new upper limit of 0 05″ has been obtained for the angular size of Cyg X-3, together with an improved position for the source

In September 1972 radio emission from Cyg X-3 increased by three orders of magnitude in the course of a few days, reaching a maximum of 22 Jy only to decline again during the following week[1] Since then some 17 outbursts have been recorded by individual observers and by a monitoring program at the Algonquin Radio Observatory[2] The present observations covering the period May 14 to 31 include measurements of flux density, linear and circular polarisation at two frequencies, 2,695 and 8,085 MHz The observations were obtained with the NRAO four element phase-stable interferometer at Green Bank The measured flux densities for Cyg X-3 are shown in Fig 1 with values of the spectral index $\alpha = \log (S_{8085}/S_{2695})/\log (8085/2695)$, where S_{8085} and S_{2695} are the measured flux densities at the two frequencies The flux densities are calibrated in terms of the flux densities of 3C48, 3C147 and 3C286 and have an uncertainty of ≤ 0 1 Jy or 3% whichever is the greater These three sources were also used as linear polarisation calibrators Their adopted flux densities and polarisation parameters are given in Table 1 Observations of these and other secondary flux density calibrators were interlaced with Cyg X-3 observations once every hour A more detailed discussion of the observations and their interpretation will be presented elsewhere

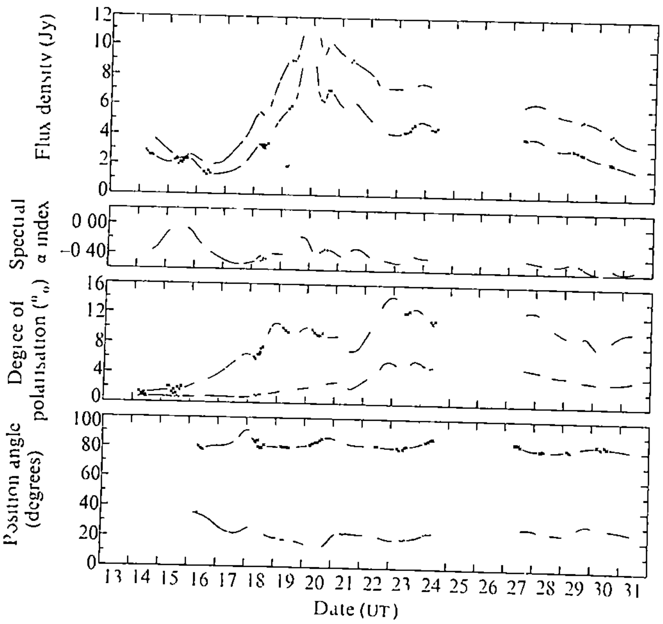

Fig 1 The flux density, spectral index, degree of linear polarisation and position angle of the radio emission plotted as a function of UT date For an unresolved source of linear polarisation P is normally expressed in terms of the degree of linear polarisation m and the position angle χ of the polarised component The measured values of m and χ, corrected for instrumental polarisation, are also shown From an analysis of the measurement uncertainties and from the constancy of the instrumental polarisation during the entire observing period, we are confident that the trends conveyed by the solid curves are real and not produced by noise or variations in the instrumental polarisation ×, 8,085 GM7, ●, 2,695 GM7 Observations cover the period May 14 to 31.

Table 1 Adopted values of flux density and polarisation for the primary calibration sources m is the degree of linear polarisation and χ is the measured position angle of the electric vector

	f=2,695 MHz			f=8,085 MHz		
Source	S (Jy)	m (%)	χ (degrees)	S (Jy)	m (%)	χ (degrees)
3C48	9 00	2 1	60	3 30	5 5	110
3C147	12 60	0 7	44	4 90	0 7	163
3C286	10 10	9 2	30 9	5 20	11 4	34 4

An attempt was made to detect circular polarisation We obtained an upper limit of 0 5% at both frequencies This limit and the theory of synchrotron radiation[3] place a corresponding upper limit of about 0 02 gauss for the magnetic field in the radiating region

The position of Cyg X-3 as measured with the maximum interferometer baseline of 35 km was found to be

$$\left.\begin{array}{l}\alpha = 20\text{ h }30\text{ min }37\ 620\text{ s} \pm 0\ 005\text{ s}\\ \delta = 40^\circ\ 47'\ 12\ 85'' \pm 0\ 05''\end{array}\right\}1950\ 0$$

This position is consistent with previous measurements[4-6] but the quoted errors of the present measurements are significantly lower A new upper limit on the angular size was obtained from simultaneous observations of Cyg X-3 with the 35 km baseline and shorter baselines over a wide range of hour angles The mean fringes visibilities measured at 2,695 and 8,085 MHz were 1 00 ± 0 03 and 1 03 ± 0 11, respectively, and no hour angle dependence was detected The minimum fringe visibility consistent with the 8,085 MHz observations corresponds to an angular size for a circularly symmetric source of uniform brightness of ≤ 0 05″ If we adopt for the distance the lower limit of 10 kpc (ref 7) this corresponds to a linear size of $\leq 8 \times 10^{15}$ cm

The radio emission observed from May 14 to 31 represents a portion of at least two large outbursts which began about May 11, 1974 (P C G , V A Hughes, A Woodsworth and E R S , unpublished) We believe that the decay in the emission between May 14 and 16 constitutes the end of the first outburst The second outburst began about May 17 The spectral index and polarisation of the radio emission together clearly indicate a nonthermal emission process characteristic of synchrotron emission from relativistic electrons If the electrons have a power law energy spectrum ($N(E) \propto E^{\gamma}$) the observed mean spectral index ($\alpha = -0\ 4$) yields $\gamma \approx 1\ 8$ throughout most of the period of the observations In addition, the spectral index profile shows a tendency for α to decline from $-0\ 2$ to $-0\ 6$ during the observations This general behaviour is characteristic of radiation from an optically thin cloud of relativistic electrons in which there are energy losses which occur preferentially at the higher energies With regard to the fact that the source is optically thin the second of these outbursts is similar to the third and fourth outbursts observed in 1972 at similar frequencies[4,8] The first two outbursts in 1972, however, exhibited an optically thick phase at and below 2,695 MHz

One of the principal differences between the May 2 outburst and all of the September 1972 outbursts is the slow rise in flux density from May 17 to May 20, followed by an even slower decline in flux density from May 20 to 31. The September activity was characterised in general by a more rapid rise and decline in the flux density. There is, however, evidence in the fine structure of the May activity that the outburst profile may be decomposed into a series of smaller outbursts, with sharp rise times, occurring so frequently that they are unresolved. The picture suggested is that relativistic particles are repeatedly injected into a region which remains essentially optically thin. On two occasions (May 15.5 and May 19.5) the spectrum flattens significantly and we presume that the associated minor outbursts correspond to particle injection in the form of a new region of emission which is either optically thick at 2,695 MHz or possesses a flatter energy spectrum in the radiating particles.

The most readily observed characteristic of the polarisation is a general increase in the degree of polarisation at both (Fig. 1) frequencies, rising from about 1% to 14% at 8,085 MHz between May 14 and May 23 followed by a slight decline. The position angle of the electric vector is remarkably constant at both frequencies, but there are significant deviations, of the order of 10°, from a mean value. The electric vector position angles at the two frequencies differ by about 60°. The latter observation leads to a mean rotation measure of about −100 rad m^{-2}. This value is consistent in magnitude and sign with the galactic Faraday rotation in the Cygnus direction, although the latter quantity is extemely uncertain. On the other hand, the deviations in rotation measure shown in Fig. 2 indicate that there is some Faraday rotation in the source as well.

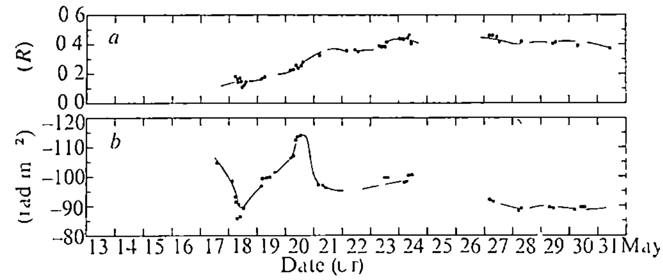

Fig. 2 The derived depolarisation ratio (*a*) and rotation measure (*b*) plotted as a function of UT date.

The degree of polarisation is lower at 2,695 MHz than at 8,085 MHz. The 'depolarisation ratio' R = (degree of polarisation at 2,695 MHz/degree of polarisation at 8,085 MHz) varies from about 0.1 on May 17 to about 0.45 on May 24 and remains roughly constant thereafter. The most direct interpretation of this result is that the radiation at 2,695 MHz is partially depolarised by Faraday dispersion produced by an ionised thermal plasma associated with the source. The polarisation observations therefore make it possible to deduce some of the characteristics of this depolarising medium.

Burn[9] has described the depolarisation behaviour of simple source models with internal and external Faraday dispersion. In particular, he computes the dependence of the observed degree of polarisation and position angle on the amount of depolarisation for varying degrees of order in the magnetic field.

We may have made a qualitative comparison of the Cyg X-3 polarisation behaviour with Burn's model for internal Faraday dispersion in the case of spherical geometry. If the model is applicable the 8,085 MHz radiation is not significantly depolarised by Faraday dispersion but that Faraday effects are partly responsible for depolarisation at 2,695 MHz. The ratio R is then a measure of the amount of depolarisation by Faraday dispersion at 2,695 MHz. So the degree of polarisation at 8,085 MHz is determined entirely by magnetic field disorder, and the position angle is determined by the field orientation. Over the observed range in the depolarisation ratio ($R = 0.1 \rightarrow 0.45$) the position angle of the electric vector at 2,695 MHz would be expected to vary by about 15° or less depending upon the detailed structure of the magnetic field. This expectation is certainly comparable with the observed slight systematic variation in the position angle at 2,695 MHz. The position angle at 8,085 MHz remains essentially fixed. If this interpretation is correct, the observed increase in the degree of polarisation at 8,085 MHz suggests an increase with time in the degree of order of the magnetic field within the source during the course of the outburst.

The observed increase in R suggests a continuous dilution of the depolarising medium with time. With regard to the internal Faraday dispersion model, this suggests either an expansion of the emitting region and depolarising medium together, or a movement of the emitting region relative to the depolarising medium.

If the ordered component of the field is primarily responsible for the depolarisation effects, then when $R = 0.45$, we have, from equations (18) and (19) of Burn[9],

$$nH_{\|}L \approx 4.7 \times 10^{14} \text{ gauss cm}^{-2}$$

where n is the thermal plasma density, $H_{\|}$ is the line of sight component of the uniform magnetic field strength, and L is the size of the emitting region.

If $L \sim 10^{15}$ cm and the field strength is about 0.01 gauss, consistent with the limits on the magnetic field derived for the first outburst[10] in September 1972, we would require an electron density on the order of 100 cm^{-3} to explain the depolarisation observed. The optical depth τ of this plasma would be about 10^{-7} at 2,695 MHz if $T = 10^4$ K, and would therefore be optically thin at the observed frequencies as required.

In a reanalysis[10] of the September 1972 outbursts have shown that a thermal plasma within the emitting region plays the major role in determining the optical depth in the first two outbursts. The high plasma densities $\sim 10^7$ cm^{-3} calculated for the first two September 1972 outbursts would produce a complete depolarisation of the radio emission by Faraday effects. Indeed, most observers (see ref. 11) found only an upper limit to the degree of polarisation. The one previously reported detection of linear polarisation[1] has since been found to be accountable for by an instrumental effect. The high polarisation observed in May is consistent therefore with the fact that the outburst is optically thin.

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Distances to the sources of observed gamma-ray bursts

WE suggest that the brief, intense outbursts of hard X rays, or soft γ rays of cosmic origin which have been termed γ-ray bursts[1] are mainly, though not necessarily entirely, of Galactic origin More specifically, the γ-ray bursts reported so far[2] seem to originate in the local (Orion) spiral arm of our Galaxy, at distances of, typically, several hundred parsec If we assume that the sources radiate into 4π sr, the absolute luminosities are ~10^{39} erg per event (It may be significant that this is of the order of the predicted Eddington limit radiation from accretion disks around collapsed stars)

Although observations of the phenomenon of γ-ray bursts are well established—the first report[1] has been substantiated by numerous independent observations[3–7]—the data are by no means extensive In particular, only a couple of dozen events[2] have been confirmed by two or more independent detector systems, and less than half of these have provided useful information on source directions The directions are of critical importance to estimates of source distance, because the more direct methods, such as correlation in time or position with reported cosmic events or stellar objects of known distance, respectively, have so far failed The amount of data is unlikely to increase significantly in the next few years, so we are obliged to squeeze what we can from existing data

At present, directional information exists for 11 events Five of these have alternative positions (see ref 2), with approximately the same Galactic latitudes (because of the fortuitous orientation of the orbital plane of the satellites), but quite different Galactic longitudes The latitude distribution can be examined in order to test the isotropy of the source distributions Figure 1 shows the number of sources above a given latitude as a function of that latitude, for 10 γ-ray bursts and for 10 sources distributed perfectly isotropically (one at a very low latitude is not included, its addition strengthens an argument discussed later) The real sources seem slightly deficient at high latitudes, a result, unfortunately, of no great statistical weight

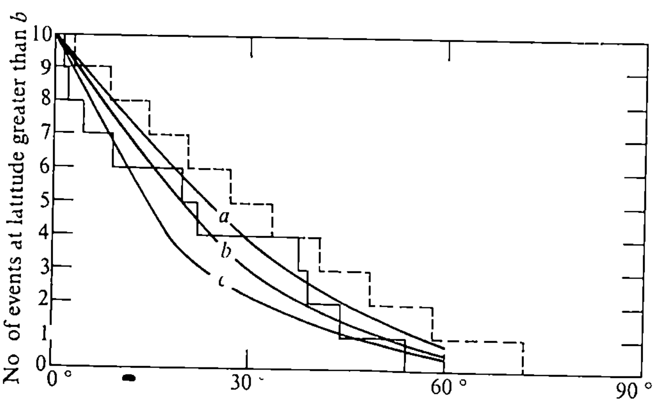

Fig. 1 Actual and isotropic integral latitude distributions compared with calculated distributions for extreme Population I objects Solid line histogram, integral distribution in Galactic latitude of 10 γ-ray burst sources, dashed line histogram, distribution expected for 10 sources distributed uniformly Calculated curves for sources of constant intrinsic luminosity, *a*, detectable out to 200 pc, *b*, 300 pc, *c*, 500 pc

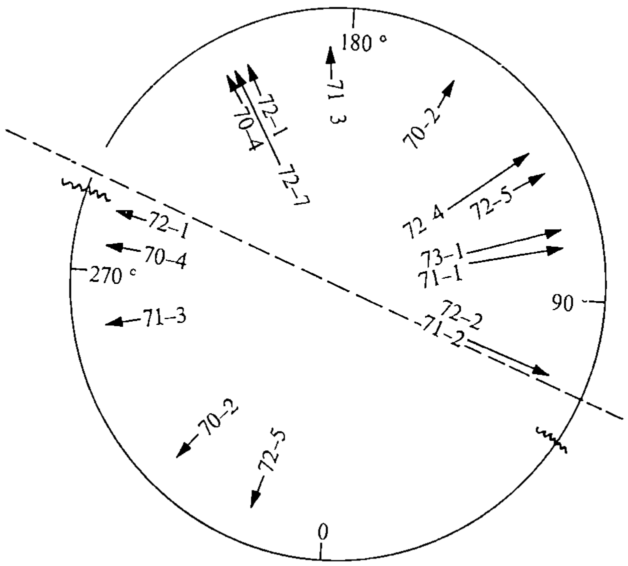

Fig 2 The distribution in Galactic longitude of 11 γ-burst sources Long arrows, unique directions, short arrows (which occur in pairs, one on each side of the dashed line), alternative positions for the source, dashed line, direction of local spiral arm The sources are named according to the system established in ref 2

The longitudinal distribution is shown in Fig 2 Here, there is something statistically more significant The orientation of the local spiral arm of this Galaxy is indicated and all six of the uniquely determined directions lie on the side away from the Galactic centre The Sun is known to lie near the inner edge of the spiral arm If the detected sources lie at distances from the Earth, which are greater than the distance of the Sun from the ill defined inner edge of the arm (a distance of, at most, a few tens of parsec) but less than the distance (around 1 kpc) to the next (Sagittarius) arm in towards the Galactic centre, then they would, if associated with the young Population I stars of the spiral arms, be expected to lie in the longitude range ~70°–~250°

Consider a simple model of source distributions in which the sources have a *z* distribution approximating that of the Galactic gas[8] and intrinsically faint stars[8] This distribution is appropriate because it is close to that of the bright O and B supergiants that have been associated with cosmic X-ray sources[9,10] Initially, we assume all the sources to have the same absolute luminosity and calculate, from the *z* distribution given by Weistrop[8], curves giving the fraction of sources above a given latitude, for any given maximum source distance (Fig 1) It can be seen that a maximum distance of ~300 pc is not a bad fit Assuming that some of the observed range of flux reflects variations in intensive luminosity, the curve can be made to fit even better, including a proportion of extra luminous sources around longitudes of ~70° and ~250°, at distances of up to ~1 kpc

An argument independent of direction can also be adduced for a Galactic origin If the sources were distributed uniformly throughout space then the number detectable above a given flux level would follow the well known '−3/2 power law' If $N(S)$ is the number of sources producing a flux greater than S then $N(S) \propto S^{-\alpha}$, where $\alpha = 3/2$ For sources confined to an infinite, thin sheet, $\alpha = 1$, whereas for sources strung out along an infinite line, $\alpha = 1/2$ This argument does not require the sources to have uniform, intrinsic luminosites

Cline and Desai (unpublished) have examined the IMP-6 data for γ-ray bursts smaller than those detected by the Vela satellites Because of a number of difficulties, the results were

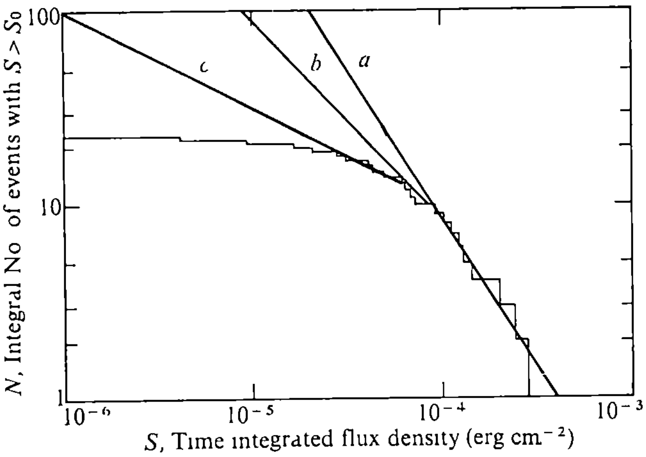

Fig. 3 A plot for 23 γ-ray bursts, detected by the Vela satellites, of the number of sources, $N(S)$, with an energy flux S or greater, against S Vertical and horizontal scales are logarithmic a, Expected curve for sources distributed uniformly throughout a volume (−3/2 law), b, source distributed uniformly over a plane (−1 law), c, sources distributed uniformly along a line (−1/2 law) (All this assumes that the detector is sensitive to infinitely small fluxes, that is, no threshold effects are present)

not conclusive, but they did report that there seemed to be a deficiency of smaller bursts—less than the number predicted by an $\alpha \approx 3/2$ If log $N(S)$ is plotted against log S for the bursts detected by the Vela satellites, the curve of Fig 3 is obtained It was at first assumed that threshold effects in the detector trigger logics could explain the break away from the −3/2 power law below $\sim 10^{-4}$ erg cm^{-2}, but a more careful examination of the problem now suggests that the deviation is mostly real If the sources are Galactic the log $N \sim$ log S curve (less a multiplicative constant) can be predicted from a simple count of, for example, O-associations or H II regions, which would have a similar distribution The resultant curve matches well the observed curve of Fig 3 down to $S \approx 3 \times 10^{-5}$ erg cm^{-2}, where the trigger threshold effects begin to predominate The curve below $\sim 7 \times 10^{-5}$ erg cm^{-2} actually follows a line with α less than 1, but above 1/2 This is partly a result of the narrowness of the spiral arms and partly because of reduced stellar density further from the Galactic centre

Two γ-ray bursts, events 71–2 and 72–2 (see ref 2) each had one of their two possible source directions very close to that of Cygnus X-1 (I B Strong, *et al*, unpublished) If this tentative identification is correct it agrees well with the argument for a Galactic origin The distance, 2 5 kpc, is about a factor of three greater than may have been expected, but if γ-ray bursts do originate in binary systems of the Cygnus X–1 type (a possible black hole?) it may be that Cyg X–1 itself is a particularly luminous member of its class

In conclusion, we warn against the automatic assumption that all the γ-ray bursts have similar origins It may be so, but we are really discussing cosmic X-ray and γ-ray emission processes of brief duration and there may well be more than one such process Also, although we consider the argument for a galactic origin to be sound, there may be a small proportion of extra galactic events included in the data

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Gamma radiation from the Crab Nebula above 35 MeV

OBSERVATIONS of electromagnetic radiation from the Crab have been made to at least 1 GeV (ref 1) and possibly as high as 10^{12} eV (refs 2 and 3) The Crab is unique among strong X-ray sources in that the major component in the low energy range (1–10 keV) shows little or no temporal variation A small ($\lesssim$ 10% at 10 keV) pulsed component from NP0532 is observed in the X-ray region, but its strength relative to the constant component increases with increasing X-ray energy Gamma ray observations of the Crab in the 15–100 MeV range have yielded rather uncertain results with reported upper limits[4,5] and fluxes[6-10] in apparent or near conflict As the reported intensities were just marginally detectable above atmospheric background with the balloon experiments which produced the observations, this seems understandable

Here we report observations of the Crab above 35 MeV made with the high energy gamma-ray telescope flown on SAS-2 The detector and technique have been described[11,12]

The data were obtained during the week of December 14–21, 1972 The Crab direction remained about 5° from the experiment axis for the entire exposure The absolute

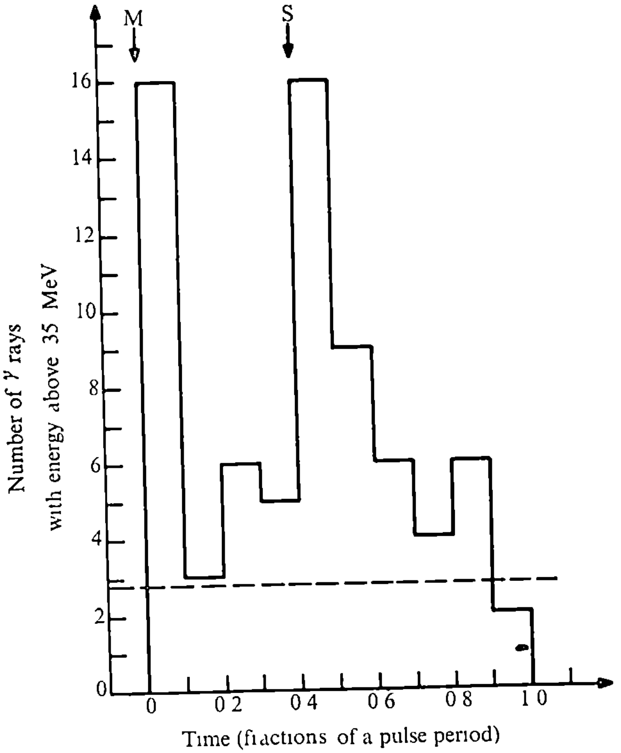

Fig 1 Distribution of γ-ray arrival time in fractions of a pulse period for γ rays above 35 MeV from the direction of NP0532 M and S refer to the predicted main and secondary pulse times as determined from radio observations (J M Rankin, private communication) — — —, background flux

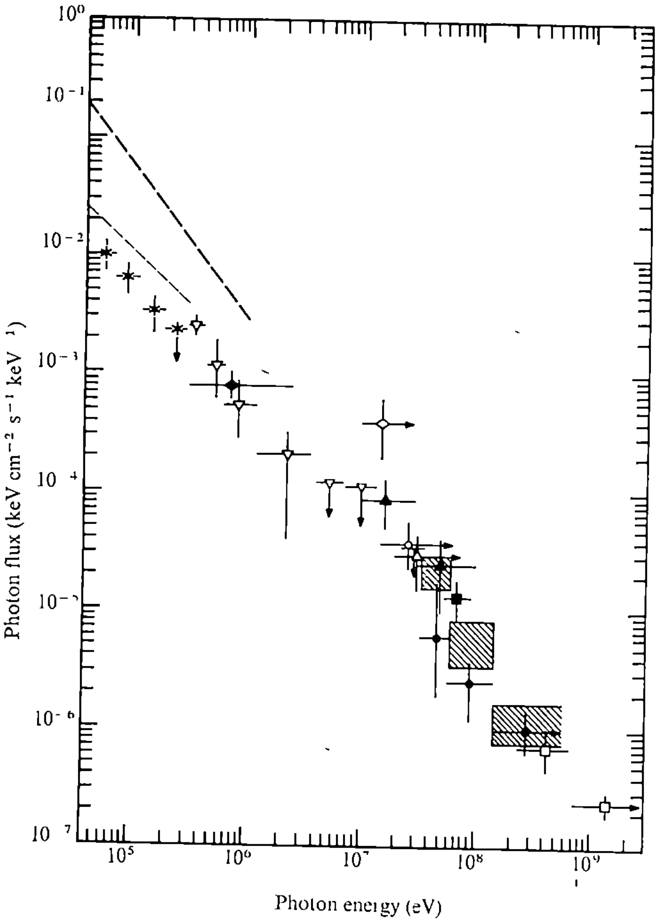

Fig. 2 Spectrum distribution of fluxes observed from the region of the Crab nebula ———, 0 7$E^{-0\ 9}$ pulsed flux[19], — — — 21 8$E^{-1\ 29}$ total X-ray flux[19], hatching, SAS-2 total flux, ●, SAS-2 pulsed flux, △, ref 17, □, ref 1, ○, ref 10, ▲, ref 7, ■, ref 6, open diamond, ref 8, △, ref 9, solid diamond, ref 18, ★, ref 14, vertical arrow, ref 5

timing accuracy of the SAS-2 satellite and the ground station was sufficient to allow separation of the pulsed component Because of the finite angular resolution of the SAS-2 detector, an angular cone of acceptance for γ rays must be included in the final analysis We used a 6° cone for γ rays with measured energies from 35 to 100 MeV and 4° for energies above 100 MeV Appropriate account was taken of the portion of the flux which is calculated on the basis of the known detector angular resolution to fall outside these circles

The arrival time of each γ ray was compared to the radio pulse time using predicted pulse arrival times (J M Rankin, personal communication) and derived as described in ref 13 (Fig 1) An independent timing analysis was made using a program based on a pulse prediction program (D C Backer, personal communication)

Peaks occur at both the positions of the main and secondary radio pulses, and there is a constant component above the general background from the Crab region Although the statistics are limited, the probability of finding by chance 16 γ rays in a bin where a peak was predicted, when the average based on the eight bins where no peak was expected is five, is less than 10^{-4} The probability of finding peaks of the observed size in both bins is then extraordinarily small As the absolute timing uncertainty for SAS-2 was about 1 ms, selecting markedly smaller intervals of time than those shown in Fig 1 does not produce peaks of increased significance

The total flux of γ rays above 35 MeV is estimated to be $(15\ 8\pm4\ 2)\times10^{-6}$ photons cm^{-2} s^{-1}, and that of the pulsed component, using bins 1 and 5 only (Fig 1) is estimated at $(6\ 2\pm2\ 8)\times10^{-6}$ photons cm^{-2} s^{-1} The total flux above 100 MeV is $(3\ 2\pm0\ 9)\times10^{-6}$ photons cm^{-2} s^{-1}, and the pulsed component above 100 MeV is $(2\ 2\pm0\ 7)\times10^{-6}$ cm^{-2} s^{-1} These results are shown in Fig 2, along with data from other experiments The unpulsed component seems to become a progressively smaller fraction of the total flux as the energy increases Above 140 MeV, only a 95% confidence upper limit of $0\ 7\times10^{-6}$ photons cm^{-2} s^{-1} can be set for an unpulsed component, whereas a finite flux of $(1\ 30\pm0\ 46)\times10^{-6}$ photons cm^{-2} s^{-1} was seen for the pulsed component The situation contrasts markedly with the low energy X-ray region, where only 10% of the radiation is pulsed (ref 14)

The most striking feature of the spectral distribution of the electromagnetic radiation from the Crab between 10^{4} and 10^{9} eV, is the shift from a predominantly unpulsed total flux at the lower energy, to one which is predominantly pulsed The spectral shape of the pulsed emission, although only poorly known, can be approximated by a power law with an energy spectral index of $\alpha\sim1$ to about a GeV Qualitatively, this picture is consistent with the Shklovsky[15] model of synchrotron emission from the speed of light cylinder with a steady flux of much lower energy photons from the region of smaller field With a magnetic field in the Nebula between 10^{-4} and 10^{-3} gauss electrons must be accelerated up to 10^{12}–10^{13} eV to produce γ rays in the 100 MeV energy region Such electron energies have been predicted in models of particle acceleration for the Crab pulsar

It is interesting to compare the γ-ray results discussed here with those for the Vela region reported by Thompson *et al*[16] The γ-ray fluxes from the two regions above 100 MeV are nearly equal, the Vela flux is $(5\ 0\pm1\ 2)\times10^{-6}$ photons cm^{-2} s^{-1} But the Crab spectrum in the γ-ray region seems to be a simple extension of the X-ray spectrum, whereas in the case of the Vela source the γ radiation is quite hard and substantially larger than an extension of the X-ray spectrum to that energy region The Vela intensity and spectra are consistent with the γ radiation being the result of about 3×10^{50} erg of cosmic-ray nuclei from the Vela supernova interacting with the local matter

It is not inconsistent with the data, for the Crab supernova also to have released a few times 10^{50} erg of cosmic rays, because as it is about four times further away, the γ radiation from cosmic-ray interactions would be approximately 16 times less intense, assuming the local matter density is not grossly different from 1–2 nuclei cm^{-3} The γ-ray intensity near 100 MeV from supernova nucleonic cosmic rays then, would be more than an order of magnitude less than the pulsed radiation at the present time

So, a consistent picture of the present data ascribes a very different origin to the γ-ray intensity from the Crab to that from Vela, with the Crab γ radiation resulting from the same mechanism responsible for the X-ray emission, and the Vela γ radiation resulting from the nucleonic component of the cosmic rays interacting with the local matter

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Gamma-ray bursts from neutron star glitches

THERE is no lack of proposed explanations for gamma-ray bursts A large amount of observational facts and theoretical arguments has been assembled recently[1] The basic facts are

- The occurrence, several times every year, of short bursts of high energy photons These events last ~ seconds and cover the spectral range between keV up to about 1 MeV
- The total flux at the earth is roughly between 3×10^{-6} erg cm^{-2} and 5×10^{-4} erg cm^{-2}
- The bursts consist of distinct components, often two in each event The time interval between components ranges from about 1 s up to about 30 s (a much more rapid intensity variation exists within each component)
- The angular distribution is roughly consistent with isotropy

The sources are either extragalactic or fairly local (say, within a few hundred parsecs) An analysis of the data now available yields a relationship for the 'number of courses as a function of intensity' which has been interpreted as favouring a local origin[2]

Here we propose that the cosmic gamma-ray bursts arise from the very abundant population of old, slowly spinning neutron stars which are no longer observable as pulsars It is supposed that the bursts are emitted because of magnetospheric activity related to glitches

The number density of defunct pulsars in the solar neighbourhood has been estimated to be around 3×10^{-2} pc^{-3} (ref 3) An order of magnitude more of these objects would still be compatible with the Oort limit 0 2 M⊙ pc^{-3} Within 10 pc one can expect 10^2 and possibly 10^3 old neutron stars

A typical burst of order 10^{-4} erg cm^{-2} implies a source power 10^{34} R^2_{pc} erg (R_{pc} is the distance in parsecs) The observed frequency of several events per year and their intensities are compatible with the number of defunct pulsars if each of them emits, say, 10^{35} to 10^{36} erg every 10 to 100 yr

This possibility is suggested by recent observations of period irregularities in old pulsars R Manchester and coworkers have monitored half a dozen pulsars during four years and have definite evidence for two irregularities ('glitches') In the first case (PSR1508 +55) they have found a glitch of relative magnitude $\delta=\Delta\Omega/\Omega\gtrsim2\ 2\times10^{-10}$ (ref 4), in the other case (PSR0329+54) the glitch is of order $\delta\simeq1\ 8\times10^{-10}$ (Manchester, private communication) These observations suggest a rough figure of about one glitch per pulsar every 10 to 20 yr

A considerable fraction of the energy released in a glitch probably causes a sudden change of the magnetospheric fields and leads to the production of fast particles How much energy is actually released is, however, model dependent If a glitch is a sudden change in stellar shape caused by internal relaxation processes the stellar magnetic field (well anchored in the conducting surface) will change by an amount $\Delta B=\delta\, B$ This will give rise to an induced electric field in the conducting magnetosphere, to Alfven waves and to particle acceleration The total energy release is of the order $\delta\,(\mathrm{B}^2{}_0/4\pi)(4\pi/3)R^3$ Typical parameters $R=10$ km $B^2{}_0=10^{26}$ erg cm^{-3}, $\delta=2\times10^{-10}$ correspond to an energy release around 10^{34} erg Note, however, that this is a lower limit because some surface rearrangements can alter B without changing the stellar moment of inertia

In alternative models the glitches are caused by magnetospheric instabilities and the energy release can be much larger

In this type of model the maximum glitch would have a magnitude[5]

$$\delta_{\mathrm{max}}=(\Delta\Omega/\Omega_{\mathrm{max}})\simeq(B^2{}_0\,R/M\Omega^2)$$

A glitch of intensity δ would release an energy of the order

$$(\delta/\delta_{\mathrm{max}})\,B^2{}_0\,R^3\simeq\delta M\,R^2\Omega^2$$

If $\delta=2\times10^{-10}$, $P=6$ s, a typical neutron star would release about 4×10^{35} erg

If the magnetic field is around 3×10^{12} gauss and $\delta=2\times10^{-10}$ the induced electric field is sufficient to impart relativistic energies to the magnetospheric particles Indeed

$$\int E\,\mathrm{d}t=R/c\ \Delta\ B\simeq10\ \mathrm{V\ cm^{-1}\ s}$$

The energies which can be reached are clearly insufficient to radiate high energy photons through the curvature radiation mechanism On the other hand, any amount of energy pumped into motions transverse to the field lines will be radiated away Low energy electrons radiate near the Larmor frequency—field measured in units of 10^{12} gauss) Relativistic electrons they emit photons $\mathrm{k}h\nu\simeq10\,B_{\perp,12}$ eV ($B_{\perp,12}$ is the perpendicular could easily emit gamma rays These high energy photons, however, would not be able to escape from the star surroundings if their energy ε (in MeV) satisfies the relationship $\varepsilon\ B_{\perp,12}\gtrsim4$ (they would instead give rise to electron–positron pairs)

If the bursts arise near the neutron star surface and the emission is not highly collimated with the field lines, our model naturally accounts for the spectral range covered by the bursts and predicts a sharp cutoff around 1 MeV

Pulsars turn off after their periods lengthen to about 3 s, at a typical age $t\simeq10^8$ yr If the torque remains unchanged, then $P\propto$ (age)$^{1/2}$ and the dead pulsars should have periods in the range from 3 to 30 s (most of them will have the slowest rotation) If, however, the braking and the radiation mechanisms are associated, (for instance if both of them result from the current flow through the polar surface) most dead pulsars would have periods not much greater than that at turnoff

These considerations suggest a possible interpretation of the time structure of the bursts If the emission arises in a specific sector of the magnetosphere and the activity lasts longer than one rotation period, then the bursts can be expected to disappear from our view because of rotation and should reappear some time later (say, 1 s up to about 30 s later) If there were no evolutionary effects in the active region there would be an exact periodicity for the repetition of pulse groups within an individual event In real life such an explosive phenomenon is likely to be accompanied by spreading and motions of the active region this would prevent genuine periodicities Rotation could also introduce other horizon effects and possibly explain why some bursts are detected not during their rise but when they already have peak intensity

Finally, we note that the neutron star model for bursts can potentially suggest the presence of two characteristic time scales The first is in the millisecond range and represents the time for an Alfven wave to cross the magnetosphere The other is roughly around 10 s, the time needed by an Alfven wave to cross the star interior

It is easy to see that the lack of gamma-ray bursts in coincidence with the glitches in the Vela and in the Crab pulsar does not pose a serious problem for our model Indeed, because of the very large distance of these sources, a gamma-ray burst would have been detected only if the power was enormously greater than in the other cases It is of obvious interest to monitor the timing of nearby pulsars this could give hope of detecting a coincidence between glitches and gamma bursts

To conclude, we note that there is no reason to exclude the possibility of glitches in neutron stars also occurring when they accrete mass from a nearby companion It is then possible that some X-ray binaries may emit gamma-ray bursts If Cyg X-1 is confirmed to be a source of bursts (I Strong, private communication) our model would require the accreting object to be a neutron star rather than a black hole

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Note added in proof. After this paper was submitted, M R received a preprint from A I Tsygan (Leningrad) containing similar ideas

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Pulsed 10–30 MeV gamma rays from PSR0833-45

Of the more than 100 known pulsars the one in Vela, PSR0833-45 is the most like the Crab pulsar in many of its characteristics It has the second shortest period (89 ms), the second largest d*P*/d*t*, undergoes sudden 'glitches' or decreases in its period[1] and is associated with a known supernova remnant[2] The many optical searches for pulsed radiation have so far been negative[3] In the X-ray region a positive result was reported for 0 5 to 1 5 keV but 29 ms earlier in phase than the radio frequency pulse[4] At 23 to 80 keV evidence was found for pulsed emission with a period 155 ns different from the radio period[5] Later X-ray observations have not detected any pulsed radiation[6–8] Observations from Uhuru show an X-ray source at this position[9], but the time resolution was too long to detect pulsing Grindlay *et al*[10] have reported a possible indication of pulsed emission above 3 × 10^{11} eV

Here we report detection of pulsed emission from PSR0833 at 10–30 MeV A sharp peak is observed coincident with the radio pulse There is also some evidence for precursor emission

The balloon-borne spark chamber detection system is the same as used earlier for the detection of γ rays from NP0532 (ref 11) On flight 571 from Longreach, Queensland, on November 16, 1972, Vela was observed[12] from 1620 to 2320 UT An azimuthal orientation system kept the spark chamber axis pointed south at a zenith angle of 35° The timing system was essentially the same as used previously except for a telemetry transmitter with a narrower passband, which caused a greater uncertainty in absolute time An on-board clock was synchronised with the ground station several hours before launch and read out for each spark chamber event The synchronisation was continuously monitored during the seven hour observation by checking the phase of the on-board clock one pulse per second against the station clock Loran-C sky wave transmission (100 kHz) from the Yap Island station was used to monitor the absolute time of the ground station clock to 0 1 ms VNG broadcasts from Lyndhurst, Australia, gave a check at 1 ms accuracy All the timing signals were recorded on analogue tape Estimated errors in the timing of events are a systematic offset of ±0 7 ms due to the instrumentation involved in comparing the on-board and ground station clocks, and a random error of ±0 3 ms for

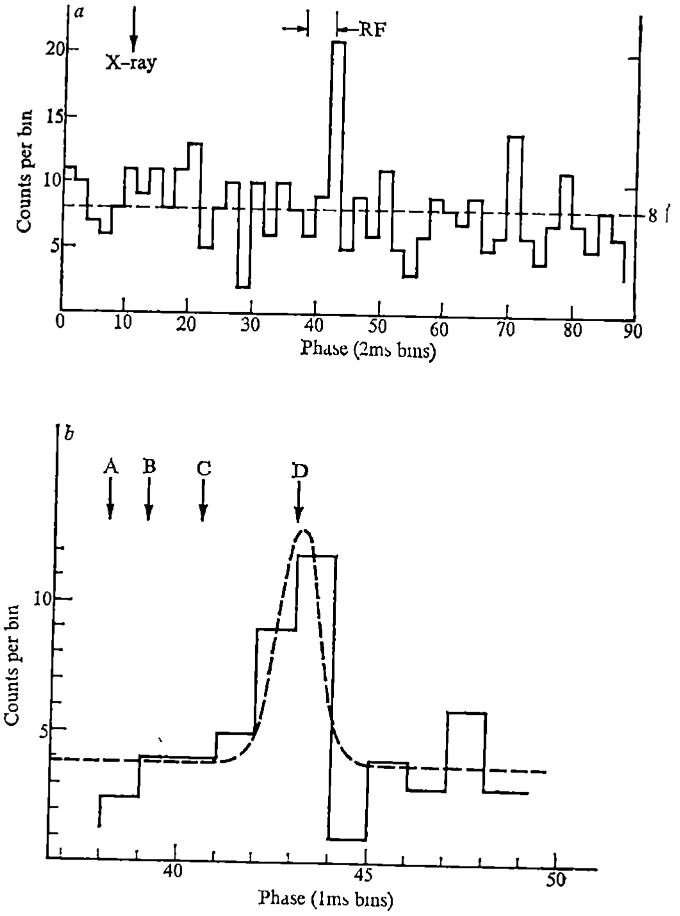

Fig 1 *a*, Phase plot of the 10 to 30 MeV γ rays from within 15° of PSR0833–45 The X-ray phase is the observation of Harnden and Gorenstein[4] *b*, Expanded phase plot of radio frequency and γ emission region showing radio timing markers A, 10% amplitude, leading edge, B, 2,388 MHz peak, C, 8,415 MHz peak, D, 10% amplitude, trailing edge ———, Maximum likelihood Gaussian (1 2 ms FWHM) the time of a given event, due to a combination of the clock 1/8 ms quantisation, uncertainty in balloon position, and variations in the electronics

Figure 1*a* shows the phase plot in 2-ms bins of the 357 events from within 15° of PSR0833 which originated in the bottom five conversion plates of the spark chamber during the 7-h exposure to Vela The Parkes group (unpublished work) monitored the pulsar during the week of the balloon flight and provided period and phase data at intervals of 12 min for the given time and location of the balloon The Parkes' phase refers to the arrival time of the half-power point of the sharp leading edge of the 1,400 MHz pulse extrapolated to infinite frequency using the dispersion measure 2 8658×10^5 s MHz^2 The absolute error on this phase is ±1 ms P Reichley of JPL made similar observations and his period predictions are within several ns of Parkes' periods The JPL phase marks the peak of the 2,388-MHz pulse The 1-ms lag of the JPL phase is consistent with the shape of the radio pulse observed at these frequencies and the reference points on this pulse chosen by the Parkes and JPL groups

At 5,000 MHz the pulse becomes double peaked (M M Komesaroff *et al*, unpublished) with a separation of ~1 8 ms between the peaks For 8,415 MHz, the later peak dominates[13] and the early peak disappears into a slight plateau region in the early part of the pulse Thus, the situation is more complicated than with NP0532 where the main pulse is a single sharp peak in the RF and maintains the same qualitative shape up through the infrared, visible and X-ray regions

Figure 1*b* shows the various timing points in more detail The maximum likelihood fit of the Gaussian shape to our unbinned data gives a peak position at 43 1 ± 0 3 ms and a width (FWHM) of 1 2 ± 0 5 ms In Fig 1*b* this comes at the 10% amplitude point on the trailing edge of the radio pulse The combined absolute timing errors do not preclude the γ-ray peak from being in phase with the 8,415-MHz pulse

The statistical significance of the peak at 42 to 44 ms in Fig 1*a* may be assessed from the following With the mean of 8 1 events per bin the chances of getting 21 or more events per bin from a random distribution during the time of radio emission (~ 6 ms) is 1 in 3,000 The statistical significance of the peak is even greater if one chooses narrower bins as suggested by the 1 2 ms width of the peak from the unbinned maximum likelihood analysis The peak is present only when events with directions near PSR0833 are chosen, and only when analysed with the unique period and change of period of PSR0833, which lends support to the identification of the peak at 43 ms as γ-ray emission from PSR0833

A further test of the reality of the effect and determination of emission against the phase of PSR0833 may be obtained by referring to the directional data The events from the lower five plates show a 2 5σ excess (independent of the phase of the events) at the position of PSR0833 This excess is modulated by the phase of the events If one takes only events with phase 0–44 (time referenced as in Fig 1*a*) the excess is 3 6σ For phase 44–88 there is no excess (101 compared with 99) We note that the Harnden and Gorenstein observation[4] of X-ray emission at ~ 1 keV occurs 29 ms before the radio emission (phase = 11 ms in Fig 1*a*) Although we see no significant narrow pulse at this position, the phase plot of Fig 1*a* has 179 events for phase 0–42 and 150 for the background phase

Thus, the 10 to 30 MeV pulse structure from Vela has similarities to that of the Crab Each shows a sharp spike ~1 ms in width coincident with the radio pulse When the poor statistics γ-ray data for the Crab are combined[15], there is evidence for about 15 ms of enhanced emission following the main pulse when compared to the next 15 ms For PSR0833 our data raise the intriguing possibility that a similar enhancement occurs for the 40 ms before the main peak

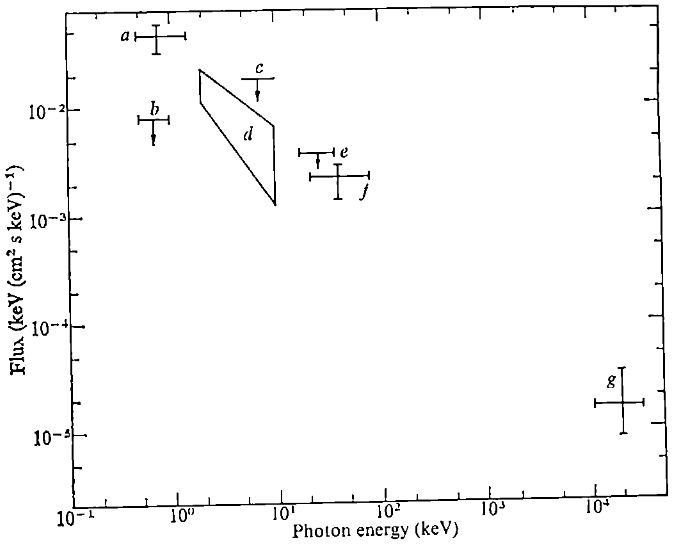

Fig 2 Spectral data on time averaged flux from PSR0833-45 above 0 5 keV All the points and upper limits are pulsed measurements except Uhuru (ref 9) *a*, ref 4, *b*, ref 8, *c*, ref 6, *d*, Uhuru[9], *e*, ref 7, *f*, ref 5, *g*, present work

The flux in the main peak to within a factor of two is 1 5×10^{-5} MeV cm^{-2} s^{-1} MeV^{-1} at 20 MeV compared to our result of (6±3)×10^{-5} MeV cm^{-2} s^{-1} MeV^{-1} for NP0532 Sturrock[14] predicts a $T^{-4\ 25}$ dependence for the γ-ray luminosity of the pulsar on its period, that is L(Crab)/L(Vela)= 68 Taking the Crab[16] and Vela[2] distances as 2 0 and 0 5 kpc our measurement is 64±32 (The intensity ratio is known more accurately than the absolute intensity of either source and its uncertainty arises primarily from the statistics and the threshold setting of the Čerenkov detector) It will be of interest to test further the Sturrock period-luminosity dependence by searching for γ-radiation from other short period pulsars with the COS-B satellite to be launched next year or the larger area balloon detectors now under construction

Figure 2 shows the existing results on the PSR0833 energy spectrum They are consistent with the spectral index of −0 9 observed for the main pulse of the Crab over the same energy range[11], but not with the −0 5 dependence predicted by Sturrock for electron polar zone radiation[14] If the excess in bins 0–42 (Fig 1) represents an actual pulsed component, the total pulsed emission (to within a factor of two) would be 4×10^{-5} MeV cm^{-2} s^{-1} MeV^{-1} at 20 MeV

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Collimation of auroral particles by time-varying acceleration

IT is generally accepted that charged particles aligned with the magnetic field, which occur in some forms of auroral precipitation[1-4], are collimated within a few thousand kilometres of the Earth's surface, by an acceleration parallel to the geomagnetic field Static electric fields are thought to provide this acceleration, and various modes of injection of charged particles have been invoked to account for the details of observed energy spectra and angular distributions[1-4] A time-varying acceleration of an incident stream of charged particles, provides an alternative explanation

We assume that the incident stream of charged particles has a Maxwellian energy spectrum Such spectra are commonly observed in the plasma sheet[5]—the probable source of most auroral particles—and the energy distributions of auroral particles often resemble accelerated Maxwellian distributions We also assume that the intensity of the incident particles is isotropic, and that there are no upward-moving particles The intensity at energy E_1 and pitch angle α_1, is then

$$J_1(E_1, \alpha_1)=A(E_1/E_0)\exp[-E_1/E_0] \text{ for } 0\leqslant\alpha_1\leqslant 90^\circ \qquad (1)$$
$$=0 \qquad \text{for} \quad \alpha_1>90^\circ$$

where A is a constant, and E_0 is the energy equivalent to the temperature of the Maxwellian distribution

We shall first consider the energy, Δ, gained by a particle traversing an acceleration region to be constant, positive and independent of energy The energy E_2, and intensity, $J_2(E_2, \alpha_2)$, of the same particles measured at a detector beyond the region, follows from the energy gain and conservation of phase-space density, along a trajectory (ref 6, and E C Ray, unpublished) Thus

$$E_2=E_1+\Delta \qquad (2)$$

and

$$J_2(E_2, \alpha_2)/E_2=J_1(E_1, \alpha_1)/E_1 \qquad (3)$$

The pitch angle, α_2, follows from an assumed conservation of magnetic moment

$$(E_2\sin^2\alpha_2)/B_2=(E_1\sin^2\alpha_1)/B_1 \qquad (4)$$

where B_1 is the magnetic field strength where the particles enter the acceleration region, and B_2 is the magnetic field strength at the detector Combining equations (1)-(4) we have

$$J_2(E_2,\alpha_2)=A(E_2/E_0)\exp[-E_2/E_0]\exp[\Delta/E_0] \text{ for } \alpha_2\leqslant\alpha_c \qquad (5)$$
$$=0 \qquad \text{for } \alpha_2>\alpha_c$$

$$\text{where} \quad \sin^2\alpha_c=(B_2/B_1)(1-\Delta/E_2) \quad \text{for } \Delta\geqslant E_2(1-B_1/B_2) \qquad (6)$$

$$\text{and} \quad \alpha_c=90^\circ \qquad \text{for } \Delta\leqslant E_2(1-B_1/B_2)$$

These results have been discussed in detail by several authors (refs 1–3, and F W Berko, and R A Hoffman, unpublished), and the main features are that accelerated particles have energies of Δ or more, and are confined to pitch angles of α_c or less, the intensity being independent of pitch angle in this range Equation (6) shows that α_c increases with increasing E_2 and B_2/B_1, and that maximum collimation ($\alpha_c=0$) occurs for particles of energy Δ Some particles back-scattered from the atmosphere would be reflected in the acceleration region and contribute to the emerging stream energies and pitch angles 'forbidden' to the particles which have traversed the region[13]

As an alternative to constant acceleration, we postulate that Δ varies with time, with the probability of occurrence, $P(\Delta)$, of a particular value given by the normal distribution

$$P(\Delta)=(1/\sigma\sqrt{2\pi})\exp[-(\Delta-\mu)^2/2\sigma^2] \qquad (7)$$

where μ is the mean value of Δ, and σ the standard deviation We assume that significant changes occur in Δ within the resolving time of our detector (10^{-2} s) so that only an average effect is observed The time scale of changes in Δ must, however, be greater than the local gyroperiod (near the Earth $\sim 10^{-6}$ s for electrons) for the magnetic moment to be conserved Similar considerations apply to the scale size If changes in Δ were accompanied by scattering, phase-space density would not be conserved and a different treatment would be required For this analysis we assume, however, that equations (3) and (4) are valid, so that the observed intensity, $J_2(E_2,\alpha_2)_{obs}$ is the average of $J_2(E_2,\alpha_2)$, given by equation (5), for all values of Δ

$$J_2(E_2,\alpha_2)_{obs}=A(E_2/E_0)\exp[-E_2/E_0] \int_{-\infty}^{\Delta m}\exp[\Delta/E_0](P(\Delta))\mathrm{d}\Delta \qquad (8)$$

where $\Delta_m, =E_2(1-(B_1/B_2)\sin^2\alpha_2)$, is the largest value of Δ making a nonzero contribution to the integral, which follows from equation (6) Substitution for $P(\Delta)$, and integration gives

$$J_2(E_2,\alpha_2)_{obs}=\tfrac{1}{2}A(E_2/E_0)\exp[-E_2/E_0]\exp[\mu/E_0+\sigma^2/2E_0{}^2](1+\operatorname{erf} x) \qquad (9)$$

where

$$x=[(E_2/E_0)(1-(B_1/B_2)\sin^2\alpha_2)-(\mu/E_0+\sigma^2/E_0{}^2)]/2(\sigma/E_0) \qquad (10)$$

When $\mu=0$ and $\sigma=0$ the particle stream is unchanged as equation (9) reverts to equation (1), when μ is nonzero and $\sigma=0$, equation (9) reverts to equation (5), the equation for acceleration by a static electric field On putting $\mu=0$ with σ nonzero, random fluctuations in Δ produce a net change in intensity, but in this case equation (9) does not revert to equation (5) Equation (9) shows on inspection that the intensity increases monotonically towards smaller pitch angles (except when $\sigma=0$) for any value of E_2 A particularly interesting property of equation (9) is that the postulated fluctuations in Δ

can, under some circumstances, collimate both electrons and protons at the same time An increase of the ratio B_2/B_1 will reduce the degree of collimation

Using a sounding rocket flown during the expansion phase of an auroral substorm, we observed electrons collimated towards the direction of the local geomagnetic field Electron energy spectra were measured three times per second over the range 0 5–10 keV, within a pitch angle range 5°–25° scanned in a 12-s period In addition, electron intensities at 6 keV were measured every 10^{-2} s, within a pitch angle range of 20°–70° scanned every 2×10^{-1} s Electron intensities were found consistently to increase monotonically towards small pitch angles at energies both above and below the peak of the energy spectrum

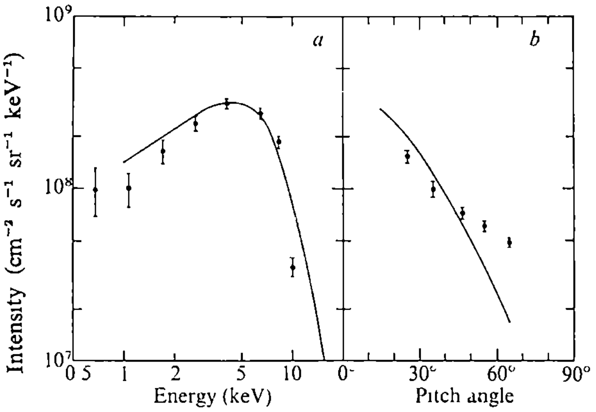

Fig 1 *a*, Energy spectrum at a pitch angle of 20°, *b*, simultaneous pitch angle distribution at 6 keV Measurements taken at 20 11 24 UT, on November 22, 1971, at an altitude of 135 km, using ESRO sounding rocket C78/2, launched from Kiruna, Sweden Curves predicted using equation (9), with parameter values $\mu=0$ keV, $\sigma=4$ keV, $E_0=1$ keV, $A=8\times10^7$ cm^{-2} s^{-1} sr^{-1} keV^{-1}, $B_2/B_1=1$ 33 The ratio B_2/B_1 was chosen assuming that acceleration took place within 700 km of the atmosphere

Figure 1*a* shows an observed energy spectrum, with a peak intensity at 5 keV and Fig 1*b* shows the simultaneously observed angular distribution at 6 keV, typically, the intensity increases monotonically towards small pitch angles Only 10% of the increase between 65° and 25° can be accounted for by atmospheric absorption This was evaluated using an identical payload in a control experiment performed under stable auroral conditions, and is consistent with calculations made by Walt[7] The absence of sharp changes in the slopes of the energy spectrum and angular distribution, together with collimation at energies below the peak of the energy spectrum are not readily accounted for by acceleration in a static electric field Either the 'back-scattered' particles would have to be collimated before reflection, which does not agree with observations[8,9], or charged particles would have to be injected or produced over a range of energies at all altitudes where the electric field was present[4] To account for our data, this injection or production would need to depend on altitude in a manner which has not been observed On the other hand, the angular distribution and energy spectrum predicted using preliminary values of the parameters in equation (9) provide promisingly good fits to the measurements shown in Fig 1*a* and *b* Equation (9) also predicts the observed collimation above and below the peak of the energy spectrum

We are unable to propose a physical mechanism which would result in the postulated distribution of the energy gained by particles A form of stochastic acceleration[10-12], or an acceleration by electric fields maintained by a time-dependent anomalous resistivity could possibly induce the effect This work was carried out as part of the research programme of the Appleton Laboratory

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Anomalous lunar plagioclase composition and the composition of coexisting melt

THE anomalous composition of lunar plagioclases may be accounted for by various substitutions, principally the replacement by Fe and Mg, with some Ca and Na, of Al in tetrahedral sites in the lattice[1]

The composition of Apollo 11 and 12 plagioclases with less than 95% anorthite becomes increasingly anomalous as the ratio Ca/(Ca+Na) decreases (Figs 1 and 2 and ref 2)

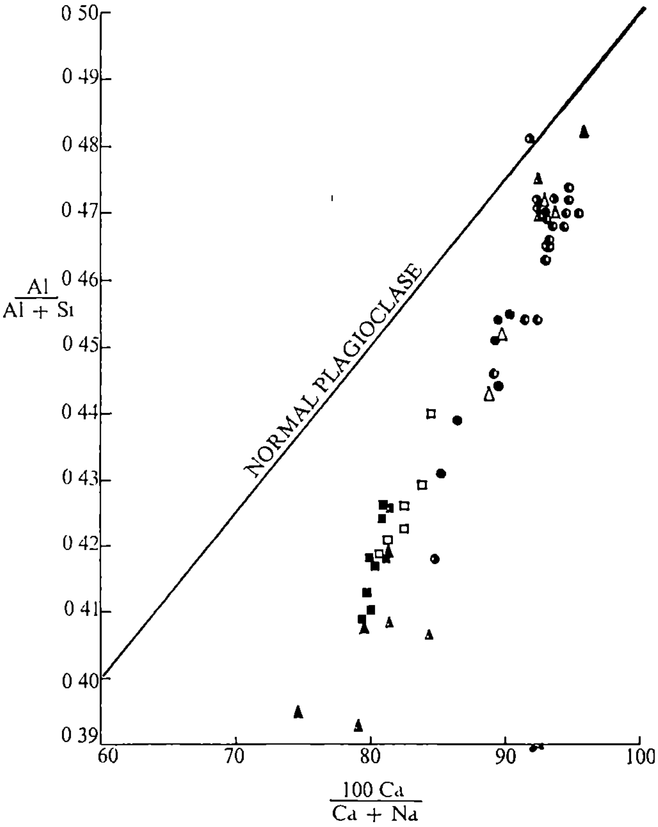

Fig 1 Al(Al + Si) against Ca/(Ca+Na) for Apollo 11 plagioclase ■, 10017 (this work), □, 10017 heated at 1,108° C for 17 h in Mo, f0_2 ≏ 10^{-13} bar (this work), △, 10045 (refs 9 and 10), ▲, 10022 (refs 11 and 12), ● ◑ ⊗, three lithic fragments in 10019*, ◐, lithic fragments in 10021*, ▲, 10062 (this work) *Analyst Mr P G Hill

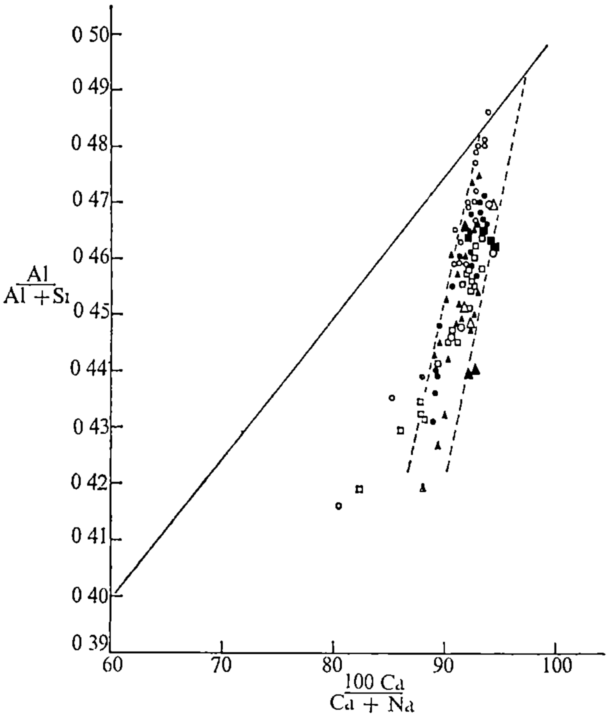

Fig. 2 Al/(Al+Si) against Ca/(Ca+Na) for Apollo 12 plagioclases △, 12040*, ■, 12040 heated at 1,149° C for 5 h in Mo, $fO_2 \simeq 10^{-12.5}$ bar (this work), ○, 12040 (this work), △, 12020 (this work), ○, 12040 (ref 13), □, 12051 (this work), ●, 12051 (ref 13), ▲, 12018 (ref 13) Plagioclases in rocks 12021 and 12004 (ref 13), 12063 (ref 14), and 12039 (ref 15), also plot within the area bounded by the dashed lines
*Analyst Mr P G Hill

Initially, the variation from the ideal composition is almost directly proportional to the albite content Rock 14310, in which all plagioclases, regardless of size or albite content, have nearly ideal compositions, (Fig 3) is exceptional Terrestrial groundmass and phenocryst plagioclases which have a similar Ca/(Ca+Na) ratio to lunar basalts, exhibit no compositional anomalies It is improbable that anomalous plagioclase is produced by the selective subsolidus volatilisation of alkalis in the lunar vacuum Plagioclase from a Reunion basalt showed negligible change in composition after heating for 1 week at 1,000° C *in vacuo* (10^{-7} torr) The relatively high albite content, and the complete exposure of the plagioclase surface to the vacuum in this experiment, would be more favourable to alkali volatilisation than is the case with lunar plagioclase, which has a generally low albite content and in which the surface of the plagioclase in the rock is either partially or totally covered

It has been suggested that one of the main factors influencing the composition of lunar plagioclase is the low viscosity of the melts which, combined with rapid cooling, gives rise to rapid disequilibrium crystal growth[1] Phase equilibria studies designed to re-equilibrate lunar plagioclases with coexisting melts indicate, however, that this is not the case Various lunar samples were heated within their melting intervals, and were then quenched rapidly (the conditions are indicated in the relevant figures) That had no effect on the anomalous nature of the plagioclase composition (Figs 1 and 2) Furthermore, there is no obvious correlation between the degree of compositional abnormality and rock texture[4] (Figs 1–3) which is assumed to reflect the cooling rate and, thus, the degree of supersaturation and rate of crystal growth

There is, however, a stong correlation between the variation from the ideal composition of lunar plagioclase and the compositions of the melts from which they crystallised This is particularly apparent with the Apollo 12 rocks (Fig 2) These show a similarity, regardless of the whole rock chemistry, between the compositions of the plagioclases and those of the coexisting melts (see Figs 3 and 4 and Table 5 of ref 3) Plagioclases with similar albite contents exhibit similar variations from the ideal composition The plagioclases from Apollo 11 rocks have not been as extensively investigated as those from Apollo 12 rocks but in support of my findings, the small number of determinations available in the literature indicates a similar correlation between the composition of the plagioclase and that of the coexisting melt[5] (Fig 1) The plagioclases in the low potassium–high titanium group of Apollo 11 rocks—10045, 10062, 10019/15, 10021/62—(refs 5–7), are similar in composition and in the degree of variation from the ideal composition, to the plagioclases from Apollo 12 rocks So in both cases there are similarities (if titanium is excluded) between the compositions of the melts which coexist with the plagioclases and the plagioclases themselves (Table 2 and analyses 6 and 11–13 of ref 5)

The maximum variation from the ideal composition corresponds to a deficiency of approximately 0.05 per unit formula in the Al+Si content, when the Ca/(Ca+Na) ratio is about 0.82–0.83 That is found in plagioclases of the high potassium–high titanium group of Apollo 11 basalts—10017 and 10022 (refs 6 and 7)—and in the rims of some plagioclases from Apollo 12 rocks—12040, 12051 It seems to be the maximum deficiency acceptable to the plagioclase lattice, because it does not increase further as the Ca/(Ca+Na) ratio continues to decrease The general trend with Apollo 11 and 12 plagioclases is, therefore, towards increasingly anomalous composition, reaching a limit in the Apollo 11 high potassium–high titanium basalts as the alkali content of the coexisting melts increases and the alumina content falls

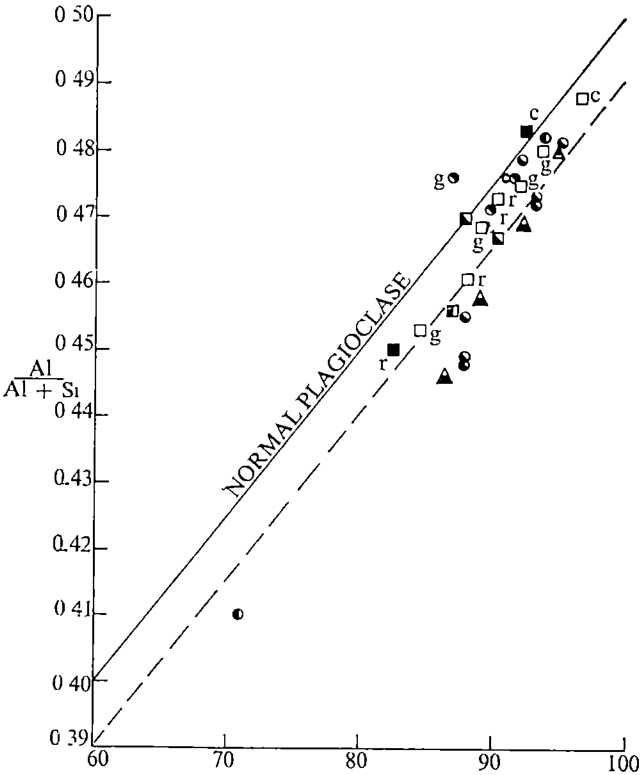

Fig 3 Al/(Al+Si) against Ca/(Ca+Na) for plagioclases in lunar rock 14310 ◐, Experimental[16], ▲, experimental[17], □, ◔, ■, ◩, ◧, ▲, plagioclase analyses from ref 18 c, Core, R, rim, g, groundmass, dashed line, maximum deviation from normal composition in terrestrial plagioclase

The importance of the alumina content relative to the alkali content of the coexisting melt in determining the degree of variation from the ideal composition is illustrated by rock 14310 The alkali contents of the melts which coexisted with the plagioclases in that rock are similar to those in Apollo 11 high potassium–high titanium basalts, but the alumina contents are much higher—30 weight % (ref 16) compared with 10 weight % (ref 5)—and the plagioclases have nearly ideal compositions (Fig 3)

If the composition of the melt is of primary importance in determining the plagioclase composition, then the same relationship should occur in lunar rocks 15555 and 15016, which have the same composition but sharply contrasted textures[8]

I have also considered whether anomalous plagioclase compositions could result because plagioclase hosts have been analysed together with submicroscopic inclusions of olivine, pigeonite, augite and silica, or liquid That would tend to increase Ca/(Ca+Na) ratio relative to the Al/(Al+Si) ratio and so the composition would vary from the ideal The areas of the plagioclase crystals which I have analysed were, however, microscopically free of inclusions If submicroscopic inclusions of olivine, pigeonite, augite or liquid are present then the extent of the compositional variation should be proportional to the Ti content of the plagioclase, which should increase as the composition becomes less ideal This was not observed Inclusions of a silica phase are unlikely to be present

The anomalous composition of lunar plagioclases probably reflects the nature of the structural groups and, therefore, the activities of the ions, particularly Al^{3+}, in the coexisting melts The influence of alkalis and/or water may be critical in this respect, and it is probable that their higher concentration in terrestrial melts is responsible for the ideal composition of terrestrial plagioclases In cases where the plagioclases have grown rapidly from melts, inherent abnormalities in composition may be exaggerated by disequilibrium crystal growth

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Earthquake prediction and oriented microcracks in rocks

VARIATIONS in the ratio of compressional wave velocities and shear wave velocities, presage some earthquakes[1] The ratio first decreases, but just before the earthquake it returns to, or rises above, the original value An attractive model has been proposed by Nur[2] Briefly, he suggested that anelastic expansion of the rock (dilatancy) reduced the velocities With the subsequent rise of the pore pressure of water, the velocity increases again The increased pore pressure also weakens the rock, resulting in an earthquake All that effects the velocity of compressional waves more than the velocity of shear waves, giving the apparent variation in their ratio

Aggarwal *et al*[3] have found, however, instances in which there are no observable variations in travel times before earthquakes They have also observed cases in which there was a clear decrease in velocity McEvilly and Johnson[4] found no variations in velocities before earthquakes in central California, although variations have been detected by Robinson *et al*[5]

In earthquake prediction it is important to understand the underlying reason for the contradictory findings Possibly, it is because of a lack of velocity anomalies in certain regions[3] McEvilly and Johnson[4] proposed that either the anomalous source regions for earthquakes on the San Andreas Fault in central California were too small, or the stress in these regions was below the level necessary to cause dilatancy Nur *et al*[6] suggested that the dilatancy hypothesis may not be applicable to strike-slip faults such as the San Andreas Although these suggestions offer useful explanations for particular cases I can suggest a unified explanation based on laboratory studies and theoretical predictions of the elastic properties of rocks containing oriented dilatant microcracks

Gupta[7] has reported experimental results on sound velocities and their ratios in a limestone subjected to anisotropic stresses oriented dilatant microcracks can be generated by clamping a rectangular specimen in one direction and increasing the difference in stress in the other two directions Gupta found little variation in sound velocities and their ratios along directions parallel to the cracks, but he found large variations perpendicular to that direction Anderson *et al*[8] showed theoretically that parallel to the microcracks the pore fluid has little effect on sound velocities and their ratios, but that it significantly affects these properties perpendicular to the microcracks

Nur *et al*[6] suggested that dilatant expansion of the crust occurs only in the direction perpendicular to the Earth's free surface That is because expansion in that direction is opposed only by gravity, whereas expansion in the horizontal directions is resisted by the surrounding rocks Although dilatancy in rock occurs at very high confining pressures, it is reasonable to expect that dilatant cracks in the crust would open predominantly in the direction of least resistance I assume the model of dilatant cracks lying parallel to the free surface of the crust The conflicting observation of the behaviour of the precursor, can then be explained

Figure 1 shows schematically a region of dilated crust with horizontal cracks, parallel to the Earth's surface Five cases are illustrated In case A, seismic waves are generated at a surface source which, together with a nearby seismic station, are located above the dilated region This situation is similar to one of the Blue Mountain Lake experiments[3] in which nearby road blasts were used as seismic sources The ray path for the seismic waves reaching the recording station penetrates the dilated crust at a shallow depth This is partly because of the surface location of the source and partly because of the anisotropy in velocity, that is, waves travelling in the horizontal direction are much faster than those in the vertical direction Therefore, the seismic rays are strongly diffracted upward Thus the waves follow a path nearly parallel to the Earth's surface, that is, parallel to the horizontal cracks So neither the seismic velocities nor their ratios will show much change as cracks open and close or as the pore pressure varies

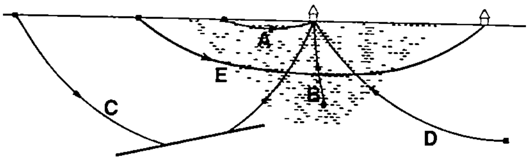

Fig. 1 Seismic rays travelling through a dilated crust Dilatant cracks are shown as broken lines parallel to the Earth's free surface A—E, see text

In case B (Fig 1), seismic waves are generated from small earthquakes in the dilated region beneath the recording station The seismic ray traverses the dilated region at a large angle to the alignment of the dilatant cracks Thus the opening and closing of cracks and changes of pore pressure in the cracks would have significant effects on the seismic velocities and their ratio The situation is similar to the Garm experiments and the Blue Mountain Lake experiments[1] Seismic rays arriving at the recording station may also traverse the cracks at a large angle to their alignment if the station is above the dilated region and if the waves are either refracted from a high velocity layer below the recording station as in case C (Fig 1) or if the waves are generated by small earthquakes outside the dilated region as in case D (Fig 1) The latter case is similar to the situations in the San Fernando earthquake of February 9, 1971 (ref 1) and the Bear Valley earthquake of February 24, 1972 (ref 5) In these cases (C and D) the velocities of seismic waves will be affected by the opening and closing of the dilatant cracks and by changes in the pore pressure in the cracks, but as the ray path in the dilated region is only a portion of the total traverse, the relative change in travel time will probably not be as pronounced as in case B Besides, in case D, the anomalous nature of source region may constitute an additional complication for the detection of the dilatant effect of the region Finally, in case E, both the recording station and the seismic sources are outside the dilated region Even though the seismic ray penetrates the dilated region it may traverse the region in a direction parallel to the alignment of the dilatant cracks Therefore, neither seismic velocities nor their ratio will show much change as cracks open and close, or as pore pressure in the cracks varies This case may be compared with the situation in the study of quarry-blast records in central California[4]

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Crystal structure of I-tetragonal boron

THERE are three polymorphs of crystalline boron α-Rhombohedral boron is the simplest form It has only one B_{12} icosahedron, the basic building block in the unit cell of all crystals rich in boron The parameters of the unit cell are $a = 5\ 057$ Å, $\alpha = 58°\ 04'$, $Z = 12$

β-Rhombohedral boron has a more stable configuration, but the crystal structure is complex[2,3] The unit cell parameters are $a = 10\ 145$ Å, $\alpha = 65°\ 17'$, $Z = 105–108$

The crystal structure of the third polymorph, II-tetragonal boron, is not known, but its unit cell parameters are $a = 10\ 12$ Å, $c = 14\ 14$ Å, $Z = 192$

Here, we discuss a non-metal boride known as I-tetragonal boron, which was first prepared in crystal form in 1943 (ref 4), and which was subsequently believed to be a polymorph of elementary boron, we have accumulated results that show unequivocally that this is not the case The crystal structure of

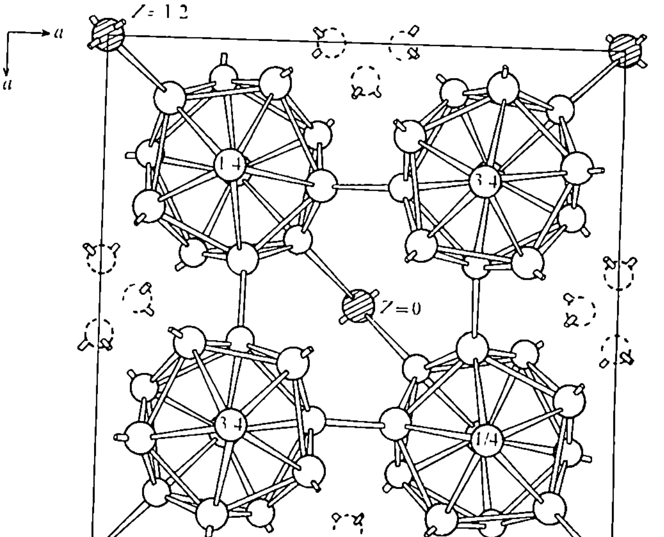

Fig 1 Unit cell of tetragonal $B_{48}B_2C_2$ and $B_{48}B_2N_2$ projected along the *c* axis and including the bonding possibilities of the single boron atoms (dashed circles) distributed statistically around 4(b) (the borons in 8(h) are omitted here) Hatched circles, carbon and nitrogen atoms

Table 1 Coordinates and occupation of the atomic sites in $B_{50}C_2$ in the space group P 4_2/nnm

Atom	Equivalent position (from International Tables)	No of electrons per site		Coordinates	
			x/a	*y/b*	*z/c*
Icosahedral atoms					
B(1)*	16(n)	5 Taken as constant	0 3259 (2)	0 0890 (2)	0 3990 (6)
B(2)	16(n)	5	0 2260 (2)	0 0806 (2)	0 0873 (5)
B(3)	8(m)	5	0 1227 (5)	0 1227 (5)	0 3798 (8)
B(4)	8(m)	5	0 2474 (6)	0 2474 (6)	0 5849 (7)
Intericosahedral atoms					
B(5)	2(a)	0 64 ± 0 08	0	0	0
B(6)	8(i)	0 49 ± 0 03	0 4330 (37)	0	0
B(7)	8(h)	0 56 ± 0 03	0 0	0 5	0 8557 (60)
C	2(b)	5 45 ± 0 07	0	0	0 5

*Standard deviations are given in parentheses

I-tetragonal boron has been discussed by Hoard *et al*[5], it has unit cell parameters $a = 8\ 75$ Å, $c = 5\ 06$ Å, $Z = 50$

We have prepared crystals of the non-metal borides $B_{48}B_2C_2$ and $B_{48}B_2N_2$, and have made precise electron density determinations of them

Quantum mechanical considerations, using the concept of closed shell type boron icosahedra[6], explain why elementary boron cannot be prepared in the I-tetragonal form Each boron atom has four valence orbitals, one 2s and three 2p In the LCAO approach the unhybridised set of 48 atomic orbitals of one B_{12} icosahedron can be combined into twelve equivalent outward pointing sp-hybrid orbitals and a set of 13 bonding and 17 antibonding molecular orbitals, separated by a substantial energy gap The latter set, with the 13 orbitals, lies mainly within the icosahedron, with strong bonding forces to the next borons on the icosahedral surface The 12 sp-hybrid orbitals are approximately perpendicular to the nearly spherical icosahedral surface, thus providing single radial sp-hybrid orbitals, each of which can form a strong σ bond between the icosahedron and the next icosahedron or any external group

To obtain a more stable crystal configuration two conditions have to be fulfilled First, to achieve a closed shell configuration, 38 valence electrons are required for each icosahedron The 12 boron atoms can, however, contribute only 36 electrons and the additional electrons have to come from intericosahedral atoms Second, to form covalent bonds, the 12 sp-hybrid bonds must find atomic orbitals close enough and in the proper directions

It is obvious, that the structure proposed by Hoard *et al*[5] with the composition $B_{50} = (B_{12})_4B_2$, cannot fulfil either of these requirements

We therefore used careful chemical preparative techniques to reproduce the crystals described by Laubengayer *et al*[4] These systematic investigations[7,8], however, yielded no results, and we concluded that it was impossible to prepare the I-tetragonal phase, $B_{48}B_2$, of elementary boron We did succeed however, in developing methods for producing the lattice of the I-tetragonal form, using pyrolysis of BBr_3–CH_4–H_2 mixtures on Ta substrates yielding boron carbides[9] This method is analogous to the method described by Laubengayer *et al* At 1,200° C a boron-rich tetragonal boron carbide of composition $B_{25}C$ (lattice constants $a = 8\ 722$ Å, $c = 5\ 080$ Å, with density $= 2\ 43$ g ml^{-1}) was deposited, and pyrolysis of BBr_3–N_2–H_2 mixtures on BN substrates at 1,400° C yielded a boron-rich tetragonal boron nitride of composition $B_{25}N$ (lattice constants $a = 8\ 646$ Å, $c = 5\ 127$ Å, with density $=$ 2 46 g ml^{-1}) (ref 10)

We have determined subsequently the single crystal structure of both the B–C and B–N phases (ref 10, and Will, G , and Kossobutski, K H , unpublished) With high precision intensity measurements we were able to determine absolute electron densities in the intericosahedral positions of the icosahedral framework using Fourier sections and least-squares techniques, reaching *R* factors of 5 8% and 5 5% for $B_{50}C_2$ and $B_{50}N_2$ respectively It was thus possible to localise and differentiate between carbon or nitrogen atoms and the single boron atoms, and to assign them to well defined sites in the icosahedral framework

The unit cell (Fig 1) contains 50 boron atoms and two carbon or nitrogen atoms Forty-eight boron atoms form four B_{12} icosahedra centred in the position 4(e) ($\frac{1}{4}\frac{1}{4}\frac{1}{4}$, $\frac{1}{4}\frac{3}{4}\frac{3}{4}$, $\frac{3}{4}\frac{1}{4}\frac{3}{4}$, $\frac{3}{4}\frac{3}{4}\frac{1}{4}$), which is in agreement with the crystal structure of Hoard *et al*[5] The remaining boron, carbon and nitrogen atoms take positions between the icosahedra in such a way that the already mentioned conditions for a stable crystal structure are fulfilled as closely as possible The resulting chemical formulae of the crystals are then $B_{48}B_2C_2$ and $B_{48}B_2N_2$

Within the icosahedra we found boron–boron distances of 1 774–1 859 Å in $B_{48}B_2C_2$, and 1 777–1 885 Å in $B_{48}B_2N_2$, with average B–B distances of 1 809 ± 0 004 Å and 1 808 ± 0 004 Å, respectively These are very similar to the values found in elementary boron[1,3], AlB_{10} (ref 11), C_4AlB_{24} (ref 12), and rhombohedral boron carbide, $B_{13}C_2$ (Will, G , and Kossobutzki, K H , unpublished)

Of the 12 icosahedral boron atoms, six form bonds with the next icosahedra, four of which have B–B distances of 1 67 Å ($B_{48}B_2C_2$) and 1 66 Å ($B_{48}B_2N_2$) Of the remaining six, two are bonded to carbon or nitrogen atoms in the position 2(b) ($00\frac{1}{2}$ $\frac{1}{2}\frac{1}{2}0$), respectively, with a B–C distance of 1 63 Å and a B–N distance of 1 59 Å, and four are bonded to two single boron atoms in the statistically occupied positions 8(i) and 8(h) The B–B distances to the atoms in 8(i) and 8(h) are 1 622 Å and 1 728 Å, respectively, for $B_{48}B_2C_2$ and 1 611 Å and 1 702 Å, respectively, for $B_{48}B_2N_2$

The 2(b) positions deserve special attention They form bridges with covalent bonding between four icosahedra These bridges are, however, occupied by carbon or nitrogen and not by boron atoms, as suggested by Hoard *et al*[5] The four icosahedra surrounding the C or N atoms form a flattened bisphenoid because the tetragonal *c*/*a* ratios are 0 58 and 0 59, respectively The boron atoms in the positions 2(a) merely fill interstitial holes They have no bonding functions

The main difference from former proposed structures is found in the two single boron atoms per unit cell—which give rise to the improvement of the electronic properties—and which are statistically distributed in the large tetrahedral holes around the positions 4(c) ($0\frac{1}{2}0$, $\frac{1}{2}00$, $0\frac{1}{2}\frac{1}{2}$, $\frac{1}{2}$) These tetrahedral holes measure about 3 4 Å in diameter and are therefore too large to accept just one bonding boron atom in a fixed position as a link between four icosahedra The borons are therefore displaced towards two of the next icosahedra, which finally yields a statistical distribution over 16 positions—8(i) and 8(h), (see Table 1) The displacement is about 0 6 Å from the centre The spatial situation easily permits such a displacement of atoms from the central position The geometrical bonding conditions in the icosahedral framework in fact force the borons to such displacements These additional atoms do, however, allow the conditions for a stable structure to be fulfilled to a very high degree

The attachment of carbon, nitrogen and boron atoms to the listed intericosahedral sites is unequivocal The quantitative occupations given in Table 1 were derived from least-squares calculations If, for example, carbon in (00½) is replaced by boron, the *R* index increases from 5 8% to 7 5%

Thus, the quantum mechanically unstable B_{48} lattice is stabilised by boron and carbon or nitrogen atoms yielding the compositions $B_{48}B_2C_2$ and $B_{48}B_2N_2$ The extra atoms fill holes in the tetragonal icosahedral framework Apart from electronic reasons the instability of the basic B_{48} lattice also results from geometrical problems which arise from the characteristic packing difficulties in three-dimensional network icosahedral structures

Further phases with the lattice of the hypothetical I-tetragonal boron include BeB_{12} (ref 13) and $AlBe(B_{12})_2$ (ref 14), and the non-metal borides $B_{50}C_2$ and $B_{50}N_2$ All can be prepared in a reproducible manner in which the tetragonal icosahedral framework is stabilised by foreign atoms For BeB_{12} and $AlBe(B_{12})$, however, no absolute electron densities are known in the icosahedral holes and no further comparison is possible

We therefore believe that the I-tetragonal boron, $B_{48}B_2$, is not a polymorph of elementary boron Hoard and Hughes[16] have already expressed doubts about the existence of this phase

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Gothenburg magnetic 'flip'

SHORT events are only recorded occasionally in stratigraphical sequences, for example, during periods of rapid sedimentation, or if the record is continuous or samples are very closely spaced If the chronology is not very exact, even these occasional records cannot usually be reliably correlated, making it difficult to distinguish between 'noise' and real events (see ref 1)

A 'Late Weichselian magnetic reversal'[2] has been found in several areas of the world We originally named[3] this reversal "the Gothenburg Reversed Event" This name, however, may not be entirely relevant The word 'event' is generally only applied to dipole reversals of durations of more than 10^3 yr The Gothenburg Reversal is a very short magnetic 'flip', which may need a term of its own, instead of 'event' Magnetic 'excursions' are defined as being caused either by dipole tilting or by dipole intensity decrease with non-dipole activity becoming dominant The Gothenburg 'flip' could be either a very short magnetic event or a magnetic excursion

The Gothenburg 'flip' was first recognised in a core (B873) taken in the Botanical Gardens, Gothenburg[2–4] The upper boundary was fixed very precisely at the boundary between the Fjarås Stadial and the Bolling Interstadial, dated at 12,350 yr BP[4,5] The change from reversed to normal polarity is very rapid (Fig 1), occurring within a couple of years Furthermore, there is no major intensity change related to the polarity change

Since we first recognised the 'flip', we have measured several additional cores and sections (Fig 2) Other workers have undertaken similar investigations elsewhere and the 'flip' has been found in several localities (Fig 2)

In the samples from localities 1–15 (Fig 2) the chronological control indicates that the recorded reversals refer to the same magnetic event or excursion, but in samples from the remaining localities the dating is not sufficiently precise for direct correlation

As yet we do not know the exact duration and form of the 'flip' There are three possibilities first, a long, constant reversal which lasted 1,000–2,000 yr (the records do not, however, show a constantly negative polarity of that duration, second, a long period (1,000–2,000 yr) of unstable geomagnetism, with several flips between normal reversed polarity, third, a short period (≤100 yr) of reversed geomagnetism at around the Fjarås Stadial (12,400–12,350 yr BP[5]) which followed a millenium of irregular (but not fully reversed) magnetism

Consequently, it may not be the proper time to decide whether the 'flip' should be classified as an event of very

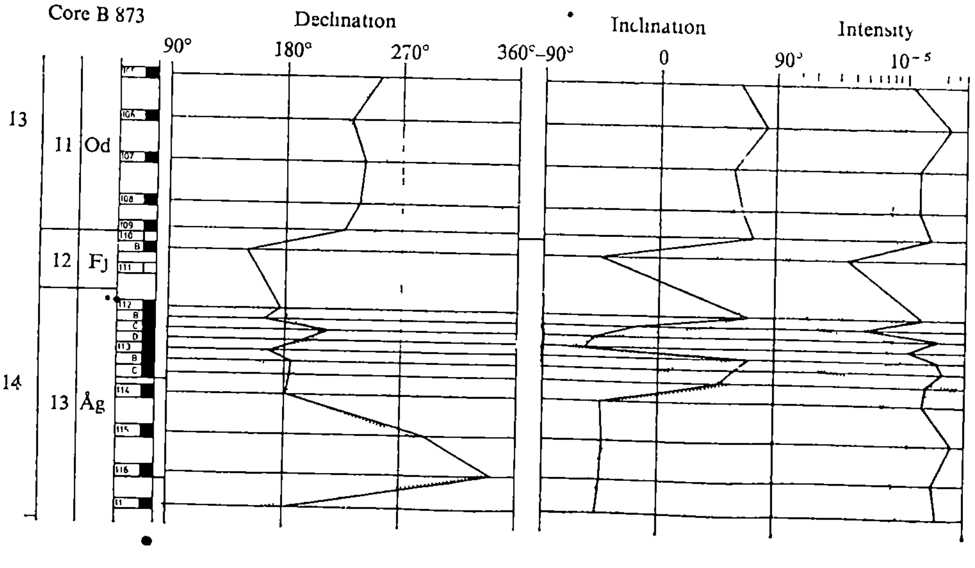

Fig 1 Palaeomagnetic measurements of the base of Core B873 (ref 4) The 'flip' includes layers 12 and 13, that is, the top of the Ågård Interstadial (Åg) and Fjarås Stadial (Fj) Solid lines, natural remanent magnetisation, dotted lines, magnetisation after cleaning in 200 oersted Layers 12 and 13 probably only represent about 100 yr

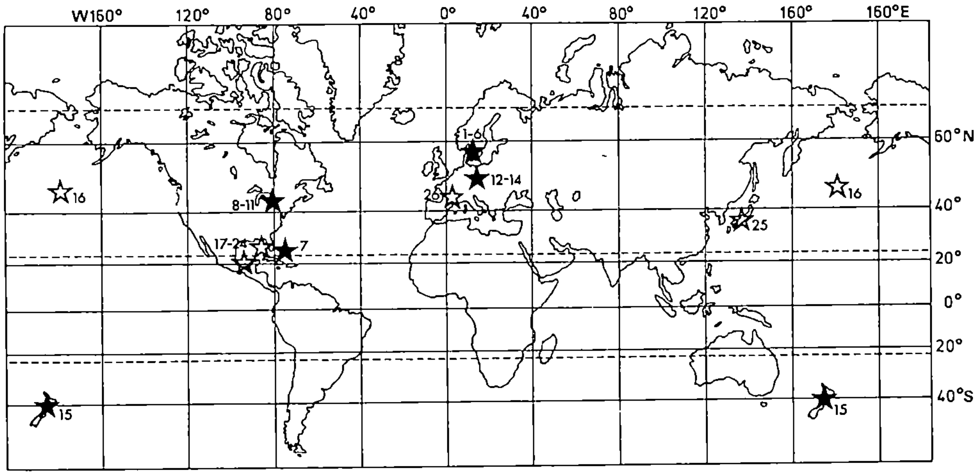

Fig 2 Localities at which the Gothenburg geomagnetic 'flip' has been established and well dated (black stars), and at which it has been tentatively recognised (open stars) 1–6, Gothenburg[2–6] and five other Swedish cores all having a well established chronology and all closely correlated on other grounds than the magnetic results (N -A Morner and J P Lanser, unpublished), 7, deep-sea core A179–15 from the North Atlantic (ref 6, and N -A Morner and J P Lanser, unpublished), 8–11, four Canadian clay sections (refs 6 and 7, and N -A Morner and J P Lanser, unpublished), 12–14, three Czechoslovakian loess sections[8], 15, the Okupata Tephra on North Island, New Zealand (W W Topping, unpublished), dated at 12,450±340 BP[9], 16, deep-sea core V20–108) from the northern Pacific[10], 17–24, eight deep sea cores from the Gulf of Mexico[11], 25, Lake Biwa-Ko, Japan[12], 26, the well known but undated Laschamp 'Event' in France[13,14]

short duration or as an excursion (or as a combination of both) The fact that it seems to be a full reversal not associated with any intensity decrease and that it is found in several different areas of the globe, seem to indicate that it is a real dipole reversal despite its very short duration

So far, the 'flip' has only been looked for carefully in a few areas In spite of that, however, it has been established in several closely dated and mutually correlated stratal sequences Therefore, it must be a real magnetic event or excursion (not just 'noises') that has global validity This means that the 'flip' is a global marker which can be used as a tool for detailed correlations on a global scale[6], and its short duration is an advantage for precise correlations

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Geostrophic comparisons using measurements from a moored current and temperature meter

THE vertical shear of horizontal current, calculated from moored current meters, can be compared with the geostrophic shear calculated from moored temperature measurements The results of this comparison show good agreement in sign and magnitude and indicate the validity of the geostrophic balance in the conservation of momentum

The geostrophic balance is used by oceanographers to infer ocean currents from measurements of temperature and salinity[1] Instruments which measure temperature and pressure at very short intervals have also been used[2] The close relationship between temperature and salinity[3] in the MODE region (28°N, 70°W) allows determinations of density from temperature measurements alone, so that moorings with several temperature recorders may serve as continuously operative hydrographic stations Current meters on the same moorings give information about the currents which may then be compared with the temperature field

In theory, ocean currents are usually assumed to be geostrophic and hydrostatic to the lowest order, implying a thermal wind relationship

$$\rho_0 f(\partial V_n/\partial z) = -g(\partial\rho/\partial s')$$

where $\vec{s}$ is the direction of the line joining two moorings, and $\vec{n}$ is the direction normal to $\vec{s}$ Assuming a close relationship between temperature and salinity, so that $\rho = \rho[T,S(T)] = \rho(T)$, the thermal wind equation becomes

$$\rho_0 f(\partial V_n/\partial_z) = g\alpha(\partial T/\partial s)$$

where $\alpha = -(\partial\rho/\partial T)$ An attempt has been made to use the MODE-I current meter and temperature data to examine this balance in its vertically integrated form

$$\rho_0 f\Delta V_n = g\int\alpha(\partial T/\partial s)dz$$

Table 1 Geostrophic comparison

	Vertical shear of horizontal current			Geostrophic shear
Time (day of year)	ΔV_n (cm s^{-1}) mooring 3*	ΔV_n (cm s^{-1}) mooring 6†	ΔV_n (cm s^{-1}) average	$(g/\rho_0 f)\int_{-721}^{-421}\alpha(\partial T/\partial s)dz$ (cm s^{-1})
100	−6 15	−4 86	−5 51	−3 26
104	−6 31	−4 82	−5 57	−5 15
108	−4 74	−4 30	−4 52	−4 87
112	−5 23	−5 38	−5 30	−5 30
116	−6 27	−4 28	−5 27	−4 82
120	−6 66	−1 66	−4 16	−4 97
124	−7 37	−5 93	−6 65	−6 13
128	−6 18	−7 58	−6 88	−5 86
132	−6 20	−5 65	−5 92	−5 56
136	−4 79	−2 39	−3 59	−4 54
140	+0 26	−0 50	−0 12	−3 30
144	−1 77	−4 85	−3 31	−3 15

* 28°09′N 70°08′W
† 28°42′N 70°16′W

In the MODE field experiment current meters at depths of approximately 425 m and 725 m on each mooring, measured horizontal velocity and temperature In addition a recorder at 525 m on each mooring measured pressure and temperature In that analysis 4-d averages of both velocity and temperature were used

Current meters on moorings approximately 60 km apart at positions 28°09′N 70°08′W and 28°42′N 70°16′W, were used to calculate the vertical change in the horizontal velocity, normal to the line joining the moorings, over the 300 m depth interval The velocity difference for each mooring and an average velocity difference for the two moorings are given in Table 1

To estimate the horizontal temperature gradient the temperature measurements had to be corrected to standard pressures, chosen at 421, 521, and 721 dbar The corrections were made using the pressure measurements and a spatially averaged CTD station, giving temperature and salinity as a function of pressure (R Milland, and H Bryden, unpublished) It was then assumed that the pressure surfaces were essentially level, so that differences of temperatures between the two moorings at each depth could be used to obtain estimates of horizontal temperature gradients, $\partial T/\partial s$ Values of $\alpha = -(\partial\rho/\partial T)$ were obtained for each depth from the average CTD station, and the quantity

$$(g/\rho_0 f)\int_{-721}^{-421}\alpha(\partial T/\partial s)dz$$

was estimated by assuming $\alpha(\partial T/\partial s)$ to be constant over a depth interval

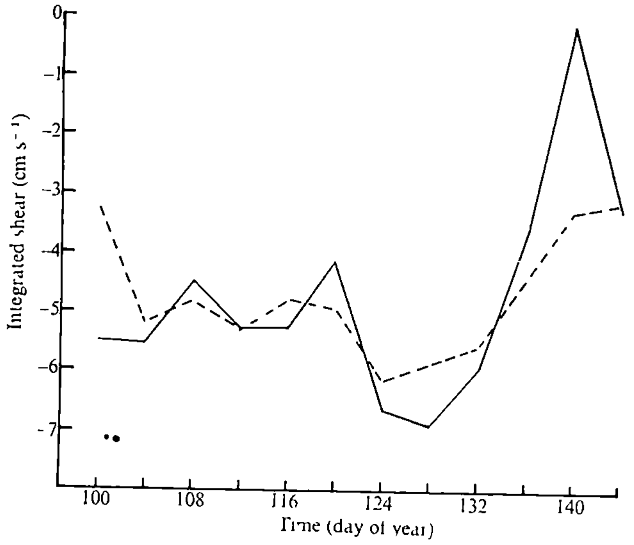

Fig. 1 Horizontal current shear, ΔV_n, from moored current meters (——), and geostrophic shear $(g/\rho f)\int_{-721}^{-421}\alpha(\partial T/\partial s)dz$ from moored temperature measurements (– – –)

Values of average ΔV_n and $(g/\rho_0 f)\int_{-721}^{-421}\alpha(\partial T/\partial s)dz$ shown in Table 1 are plotted in Fig 1 as a function of time The average difference between the observed and calculated shears is 0 01 cm s^{-1}, and the average absolute difference is 0 87 cm $^{-1}$, about 18% of the current shear

Similar comparisons were attempted using other mooring pairs All other MODE pairs with good current meters and pressure–temperature recorders were separated by horizontal distances greater than 60 km These comparisons were not as good but they did on the whole show agreement in sign, with differences in magnitude of the shears of about 3 cm s^{-1} over the 300 m vertical separation

The MODE experiment is supported by the Office of Naval Research and the National Science Foundation, The Office of Naval Research, and the office of the International Decade of Ocean Exploration of the National Science Foundation maintained the equipment and provided data analysis Dr W Schmitz and Professor C Wunsch kindly made released current meter data and pressure–temperature recorder data, respectively This work was supported by the Office of Naval Research I thank Dr N Fofonoff for encouragement

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Spatial ability, throwing accuracy and man's hunting heritage

ATTENTION has recently been drawn[1] to the indications that spatial ability—the ability to create, maintain and mentally transform a visual image[1,2]—has an X-linked recessive mode of inheritance in Caucasians[1,3-8], with males having a proportionately greater facility[1,9-12] At first glance, this result seems paradoxical in that such a presumably adaptive trait would not be expected to be transmitted on a recessive allele If past selection pressures were exerted primarily on males, however in whom the spatial allele is co-dominant, the paradox would be resolved satisfactorily

Moreover, this is an interesting focus for anthropological research because the existence of an X-linked behavioural trait in the present would constitute independent evidence for a sexual

Table 1 Means, s d s and correlations among the variables

N = 61	Spatial	Velocity	Concentric circle	Horizontal directional	Horizontal deviation	Vertical directional	Vertical deviation
Means	24 64	68 91	2 82	4 88	0 84	3 87	1 10
s d	9 93	10 32	0.52	0 38	0.25	0 47	0 29
Spatial	1 0						
Velocity	0 14	1 0					
Concentric circle	0 26	0 51	1 0				
Horizontal direction	−0 05	0 08	−0 07	1 0			
Horizontal deviation	0 0	−0 44	−0 71	−0 08	1 0		
Vertical direction	−0 11	−0 17	−0 01	0 01	−0 04	1 0	
Vertical deviation	−0 37	−0 48	−0.83	0 20	0 32	0 10	1 10

division of labour during the 99% of human history that men have been hunters Certainly, directional orientation and memory for visual landmarks must have been required for a man to find his way back to the group If, in addition, spatial ability were positively correlated with judgement of distance and throwing accuracy in males (for example, as regards such weapons as stones and spears), this could presumably have allowed them to hit game at greater distances, thereby making hunting less hazardous and resulting in longer lived hunters as well as more meat

With these ideas in mind, we have collected data on spatial ability and throwing accuracy in 67 white boys, 14–16 yr of age Data for each subject include his age, his raw score on the PMA spatial test[13] and several measurements of his average performance over 20 successive throws of a baseball at a vertical target 30 feet away[14-16] The target was a section of canvas, 9 × 9 feet, on which were inscribed five concentric circles, from 1 to 5 feet in diameter, with the centre circle or bull's eye 3 5 feet from the floor The target area was also divided by very light lines into a grid of 81 1-foot squares Five measures of accuracy were derived for each thrown ball (1) Concentric circle—with point values declining from 5 for a bull's eye to 0 for throws missing the outermost circle, (2) horizontal direction—differentiating right from left, scored 0 5 to 9 5, (3) horizontal deviation—the absolute distance from the central vertical column in either direction, scored 0 to 4 5, (4) vertical direction—differentiating above from below the central row, scored 0 5 to 9 5, (5) vertical deviation—the absolute distance from the central horizontal row in either direction, scored 0 to 4 5 In addition to the accuracy records, the speed of each throw was measured and converted to a velocity in feet per second[15,16] Subjects were allowed to warm up according to their own individual preferences, after which they were instructed to execute 20 overhand throws, in two bouts of 10 each, as fast and as accurately as possible

No significant relationship to age was found for the spatial or accuracy scores For the spatial test, which has a multiple-choice format, only the raw scores were available and we could not correct for guessing Therefore, six subjects scoring at or below chance levels were eliminated from the analysis The descriptive statistics for the remaining 61 subjects are shown in Table 1

A regression analysis was performed to assess the extent to which spatial scores were a predictor of throwing performance The results are the univariate F statistics on 1 and $N-2 = 59$ degrees of freedom in Table 2*a* Clearly spatial ability is significantly related only to the vertical deviation and concentric circle accuracy scores Proceeding a step further, we performed a step-down regression analysis in which dependent variables 1 to n are successively removed as covariates, n going from 1 to 5, with the $n+1$st variable thus being regressed on the preceding n variables and the spatial score Each of the step-down Fs then has 1 and $N-n-1$ degrees of freedom[17] This analysis indicates that when the effect due to vertical deviation accuracy is allowed for, concentric circle scores lose their relationship to spatial ability The converse is not true however When concentric circle accuracy scores are partialled out (Table 2*b*), spatial scores still predict vertical deviation scores at the 3% level of significance Thus, concentric circle scores show a relationship only because of their vertical component

This relationship between spatial ability and vertical accuracy is especially interesting in that vertical deviations on an upright

Table 2 Statistics for regression analysis on PMA spatial test

(*a*) Variable	Square mult R	Univariate F 1 and 59 d f	Chance probability	Step-down F	Chance probability
Vertical deviation	0 14	9 22	0 004	9 22	0 004
Concentric circle	0 07	4 14	0 046	0 54	0 46
Velocity	0 02	1 10	0 30	0 05	0 82
Horizontal deviation	0 0	0 0	0 98	0 38	0 53
Horizontal direction	0 0	0 13	0 72	0 14	0 71
Vertical direction	0 01	0 82	0 37	0.35	0 55
(*b*)					
Concentric circle				4 14	0.046
Vertical deviation				5 25	0 026

target would translate into skill and judgement with respect to distance if the target were laid on the ground Therefore, while emphasising that only 14% of the variance is accounted for by the PMA test, we believe we have tentative evidence relating spatial skills to an important component of the hunting enterprise Although caution is warranted in extrapolating from the present data to our hunting heritage, the suggestion of Washburn and Lancaster[18] should be noted

> "The Pleistocene way of life can only be known by inference and speculation As we go farther back in time, there is less evidence and the biological and cultural difference becomes progressively greater Yet it was in those remote times that the human way of life took shape, and it is only through speculation that we may gain some insights into what the life of our ancestors may have been "

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Local suppression of alpha activity by pattern in half the visual field

If Lippold's hypothesis[1], that the alpha rhythm of the human electroencephalogram (EEG), (and perhaps other electrical signals that can be recorded from the scalp) are artefacts that result from eye movements, rather than genuine manifestations of neural activity, was found to be correct, it would seriously reduce the value of EEG and evoked potential techniques in research and clinical neurology In the latter area, for example, considerable attention is being given to the validity of accepting an absence of EEG activity as a criterion of cerebral death One of us (C R C) has already discussed[2] earlier experiments that relate to the eye-movement hypothesis We will describe here results that are consistent with a neural explanation for the control of alpha activity, but not with an eye-movement explanation

Briefly, Lippold's hypothesis states that alpha activity is found when the standing potential between the front and rear of the eye is modulated by eye movements (especially movements that are parallel to the optic axis) and by changes in the length, and therefore in the resistance, of the extraocular muscles In addition, electrical activity of the extraocular muscles may contribute directly to the alpha rhythm According to this hypothesis, the act of observing a visual stimulus reduces alpha activity by reducing tremor of the extraocular muscles

If alpha rhythm depends on muscle tremor, visual stimulation should result in an equal reduction of alpha over both hemispheres, irrespective of which part of the visual field is stimulated If alpha is under neural control, however, the amount of suppression of alpha in one hemisphere should depend upon which lateral half of the visual field is stimulated, as, except for a small central region, the primary pathways from the two halves of the visual field project to separate hemispheres We find that visual suppression of alpha activity is related to the location of the visual stimulus

The subject faced a rear-projection screen that subtended a visual angle of approximately 90°×90° The screen could be illuminated uniformly so that very little visual detail was visible, or a pattern could be projected on the left or right half of the screen When a pattern appeared in half the screen, the subject was instructed to fixate in the blank half of the screen, about 1° from the edge of the pattern Thus, when the pattern appeared on the left, only the right visual cortex received direct stimulation from the patterned area, and *vice versa* In a preliminary experiment the pattern was a black-and-white chessboard, but the subject found this so soporific that the pattern was later changed to a series of coloured slides that were rich in visual detail (for example, interior views of the Royal Pavilion in Brighton) In another preliminary experiment, the effect on alpha suppression of varying the luminances of the patterned and blank fields was studied No difference was found as luminance was varied from that luminance at which the pattern was first clearly visible, to the maximum luminance of the projector, nor did the relative luminances of the pattern and blank fields have any measurable effect For simplicity, in the main experiment the patterns were projected at full intensity (about 200 cd m^{-2})

The subjects were four right-handed males who had normal vision and fairly abundant alpha Electrical activity was recorded bipolarly between chlorided silver electrodes in positions O_1–T_5 (above the inion, and left of centre) and O_2–T_6 (above inion, right of centre) In addition to conventional EEG recordings, the outputs of the left and right hemisphere preamplifiers were led to two bandpass filters that had quality factors of 10 The centre frequency of the filters was set at the individual subject's alpha frequency, which was measured from the EEG record while he faced the blank screen with his eyes open The outputs from the filters were rectified and led to integrators that gave the sum of activity at the alpha frequency during 1 min recording periods We used this signal, which includes both the amplitude and abundance of activity at the alpha frequency, as our principal measure of the effectiveness of selective visual stimulation

Less activity was consistently recorded from the scalp over the hemisphere that was contralateral to the visual field in which the patterned stimuli were presented Although the ratio of activity from the blank- and patterned-hemisphere derivations was never large (the maximum ratio, averaged over an experimental session, was approximately 2 1), it was highly significant, as shown by the inter

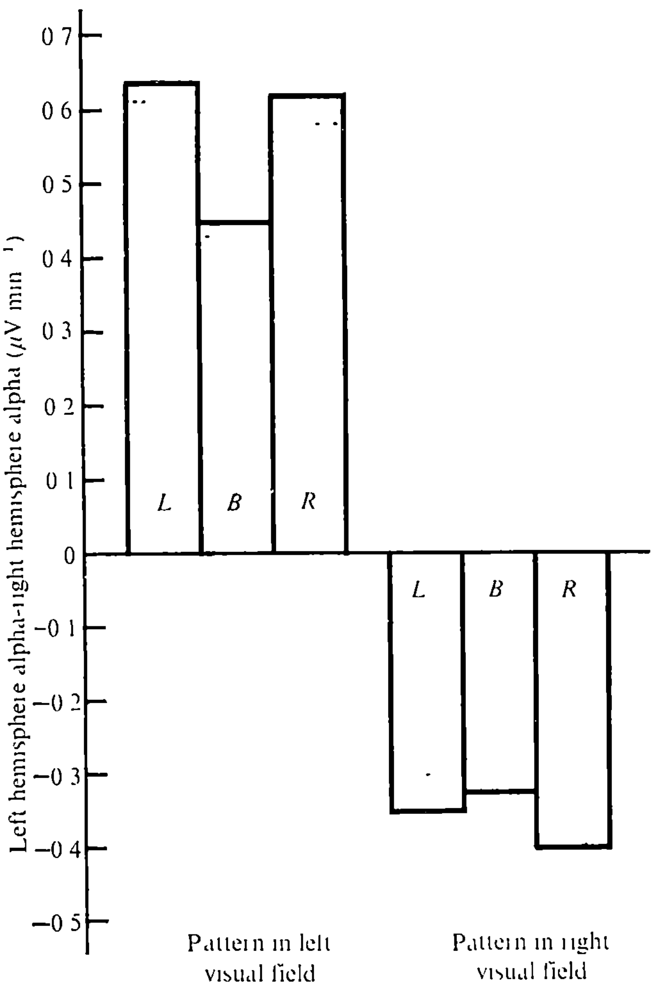

Fig. 1 Effect of left eye (*L*) and right eye (*R*) monocular observation and binocular (*B*) observation on the relative amount of alpha activity over the left and right occipital regions. The distance from the dotted line to the top of each bar = 1 s.e. The interaction between visual field and recording hemisphere was significant at $P < 0.00001$

action between visual field and hemisphere (Table 1). It should be noted that this is a conservative measure of the effect of changing the side of the visual stimulation, as any extraneous electrical activity that fell within the passband of our filters, such as movement and muscle artefacts, would have added randomly to the amplitudes of the signals from both recording sites and would have tended to obscure the difference that was produced by selective visual stimulation. Table 1 also shows that the major source of variance in the recorded alpha occurred among subjects, which is not surprising. In addition, the abundance of alpha was slightly, but consistently, greater over the left hemisphere in all subjects.

Lippold suggests[1] that an important criterion for true alpha is that its frequency remain constant. We therefore monitored the frequency throughout the recording sessions by means of a digital counter that was triggered by zero crossings. Using an average response computer, triggered in the same way, we also cumulated activity from the electrodes that were ipsilateral and contralateral to the side on which the patterned stimulus was presented. Very little change in frequency was observed during periods in which alpha-like activity could be seen in the raw signal from the scalp electrodes, and at no time was there a systematic change in frequency with change in the side on which the stimulus was presented, which leads us to conclude that the activity that we recorded satisfies Lippold's definition of true alpha.

The principal experimental evidence that has been advanced for the eye-movement hypothesis has come from studies in which differential treatment of one eye, such as dark adaptation, or cooling of the orbit, was accompanied by the appearance of asymmetrical alpha activity over the right and left occipital regions. If alpha is controlled primarily by the ipsilateral eye, it should be predicted that asymmetrical alpha will be found when a visual stimulus is presented to one eye only. To test for this, one subject observed the visual fields monocularly as well as binocularly. (During monocular observations the other eye was covered by a flap of black cardboard.) The results in Fig. 1 clearly show that alpha activity depended on the position of the patterned and blank fields, quite irrespective of which eye was stimulated. Monocular viewing with either eye resulted in slightly more suppression than binocular viewing, but we tend to attribute this to the fact that the monocular observations were made near the end of a long recording session, when the subject had more abundant alpha, and hence more alpha to suppress.

Table 1 Results of analysis of variance of alpha blocking by visual stimulation

Source	Mean square	d.f.	*P*
Visual hemifield	0.73	1,144	n.s.
Recording hemisphere	31.38	1,144	$P \ll 0.00001$
Subjects	324.07	3,144	$P \ll 0.00001$
Visual field × hemisphere	31.63	1,144	$P \ll 0.00001$
Visual field × subjects	0.77	3,144	n.s.
Hemisphere × subjects	5.43	3,144	$P < 0.01$

The main findings in this study are consistent with Morrell's observation that, during an operation in which a patient's visual cortex was exposed, highly localised suppression of alpha activity could be effected by stimulating a restricted part of the patient's visual field[3]. All these results are explained if we accept Adrian and Matthew's conclusion that visual stimulation suppresses alpha activity by means of neural activity[4]. We do not believe that they can be satisfactorily accounted for by the eye-movement hypothesis, for in our main experiment both eyes received the same stimulation and presumably would have been induced to engage in the same movements, if indeed there were any.

These findings also strongly suggest that alpha activity is controlled by visual stimulation and not, as has been claimed, by the act of fixing the gaze[5], or by rolling the eyes upward[6].

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Water in the nutrient cycle of a Papuan rain forest

INTEGRATED ecosystem studies of nutrient cycling on a catchment basis[1] for tropical rain forest areas are few[2–4], but information on the effects of nutrient cycling in these ecosystems on soil development, hydrology, and variations in stream water quality is needed, since rain forested areas in many developing tropical countries are rapidly being cleared to provide agricultural land[5].

I carried out an investigation into the hydrological part of the nutrient cycle in a tropical rain forested catchment of 16.26 km^2 below the Sogeri Plateau in Central Papua, Papua New Guinea (Fig. 1). The stream (Ei Creek) has incised approximately 600 m into the Pliocene andesitic and basaltic volcanic agglomerate capping of the plateau to expose the underlying Cretaceous phyllite, which includes chlorite, mica, and graphite, and comprises approximately 13% of the catchment. The catchment is ringed by cliffs, giving way to steep convex valley sides with a shallow covering of acid red to brown clay soils. Gullying occurs under the forest, and landslips occur after heavy rains. The rain forest of the area is dominated by *Castanopsis* and *Elaeocarpus* species which provide an open high storey, a more dense understorey, and a rich growth of ferns, epiphytes and lianes[6].

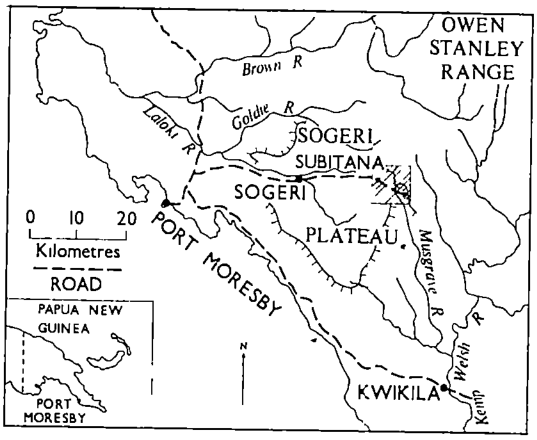

Fig. 1 Location of the study area.

A Hellman type pluviograph and a Bristol gauge recorded rainfall and streamflow for 10 months (July 1972 to May 1973), samples were taken weekly, and more often when heavy storms produced stream rises. Because of human interference throughfall could not be sampled continuously, but samples were taken, through five individual storms and an incidence of dew fall, with four 0.25 m^2 polyurethane coated troughs placed 1 m above ground. This method provides a large sample area equivalent to approximately 75 standard 13 cm diameter polythene funnels, but tends to mask the true throughfall variability. Leaf litter leachate and soil water solutions were simulated by washing with distilled water[7,8].

Water samples were analysed with a Varian Techtron atomic absorption spectrophotometer for Ca, Na, K and Mg. Samples were not buffered for Ca analysis[9], and the absence of Ca in the cation sequence (Table 1) for leaf litter leachate may be due to interference or precipitation out of solution, Ca results may therefore be anomalous.

Table 1 Cation sequences (meq l^{-1})

Rainfall	Na	> Mg	>	K	>	Ca	
Throughfall	Na	> Ca	>	K	>	Mg	
Leaf litter leachate	Mg	> Na	>	K			
Soil water (to 15 cm)	Mg	> Ca	>	Na	>	K	
Soil water (to bedrock)	Mg	> Na	>	K	>	Ca	
Stream water	Mg	> Na	>	Ca	>	K	

The colorimetric heteropoly blue method[10] was used for Si analysis. Dissolved salts and suspended solids in stream water were also measured.

The figures for the annual transfer of nutrients in the hydrological cycle (Table 2) are derived from measured elemental concentrations and measured or estimated volumes of water. Because of insufficient throughfall measurements an approximate value, of 80% rainfall as throughfall and stemflow, was arrived at by reviewing the literature for other tropical rain forests[11–13]. The chemistry of stemflow has been found to be not very dissimilar to throughfall[2,12,14], and, as it constitutes a small percentage of the rainfall reaching the forest floor, its inclusion with throughfall will not lead to large errors in estimates of nutrient return.

In simulating leaf litter leachate and soil water solutions by washing with distilled water there is the problem of accounting for residence time of water in the compartments, and the lateral flow and mixing of water with depth in the soil. McGinnis *et al*[15] calculated a residence time of 0.35 d for water in the litter layer, and 33.06 d for water in the soil of a moist tropical forest. If residence time represents leaching ability, the annual turnover of nutrients in these compartments may be very different from that calculated here. The rate of nutrient turnover in the forest floor is probably dependent upon both the volume of water and the length of time the compartment remains saturated. Not all of the 2,155 mm reaching the soil surface will reach the bedrock, and use of this volume of water in calculating the nutrient flow out of the lower soil compartment may result in an overestimate. Since the soils in this study were shallow and lateral flow on the steep slopes may add to the flow, the overestimate may not be too great. In spite of these problems, the cation sequences in Table 1 are very similar to those found in lysimeter studies under rain forest in Costa Rica[2].

The values for throughfall in the nutrient cycle indicate the rates and relative importance of the movement of individual elements in the hydrological section of the cycle. On a kg ha^{-1} basis, Na provides the greatest return in throughfall, followed by K, Ca and Mg. Neither Nye[12] nor Kenworthy[3] measured Na in throughfall, but both found the above sequence. The relative importance of throughfall in total nutrient return (litter, timber and throughfall) was shown by Nye[12] to be K, 75%, Ca, 9%, and Mg, 25%. Although actual amounts of Mg in throughfall are low, throughfall is of relatively greater importance to Mg than to Ca in their respective cycles. Both Jordan[16], and Sollins and Drewry[14] considered that in tropical rain forests nutrient return in throughfall, and in particular K, is as important as litter fall in the nutrient cycle.

Silicon was not found in rain or throughfall, but the high Si content of the forest floor is considered by Rodin and Bazilevich[17] to be typical of the tropical rain forest. Si represents a different type of cycling from the other elements considered here, it is not washed out in throughfall and depends entirely on litter and timber fall for its return to the ground, whereafter it must be leached from the litter layer in order to be returned to the roots through the soil. The low *p*H and destructive flora and fauna of the forest floor provide an ideal environment for the removal and transfer of Si. The accumulation of Si by forests[18] is of geological significance and many tropical trees have

Table 2 The nutrient cycle

	Depth water (cm yr^{-1})	Si	Ca	Na	K	Mg
		kg ha^{-1} yr^{-1}				
a, Rain	269 37	0	0	8 35	0 81	0 27
b, Throughfall	215 50	0	23 27	83 18	34.91	6 47
c, Leached from canopy	215 50	0	23 27	74 83	34 10	6 20
d, Leaf litter	215 50	13,447 20	0	6,439 14	6,661 11	5,021 15
e, Surface 15 cm soil	215 50	512 24	232 74	234 25	333 16	983 97
f, Soil to bedrock	215 50	436 39	6 47	159 04	121 54	170 68
g, Stream water	147 83	288 08	24 75	66 00	14 93	50 95
h, Total nutrients ($a+c+d+e+f+g$)	—	14,683 91	287 23	6,981 61	7,165 65	6,233 22
i, Net loss ($g-a$)	121 55	288 08	24 75	57 65	14 12	50 68

No samples *a*, 20, *b*, 49, *c*–*f*, 3, *g*, 99

been found to contain high concentrations[19] Mg dominates the cation sequence for the litter, soils and stream water This was found to be the case in McColl's study[2], and supports Kenworthy's observations[3] that most of the Mg is derived from litter leachate Litter is of great importance under tropical rain forests because of the rapid rate of decay, estimated by Nye[12] to be 1 3% d^{-1}

In stream water, Si, Mg and Na, showed strong tendencies to dilute with increasing discharge, Ca displayed a poor dilution relationship, and K concentrated weakly with increasing discharge Similar relationships with discharge have been found in widely differing study areas[20,21] which suggests that biological rather than geological processes are dominant in the variability of stream water quality The large amounts of nutrients involved in both the solid and dissolved phases of the cycle, compared with the losses in stream water (less than 1 2% of total nutrients measured), demonstrate the fully closed nature of the nutrient cycle The persistence of relatively low concentrations of elements in stream water through a wide range of discharges is due to the efficiency of the root systems in filtering out the nutrients leached through the soil The loss in stream water, however, does not include the elemental composition of suspended sediment and organic debris At peak flows after large storms much sediment and organic debris is washed into the stream from the forest floor, trees are carried downstream and the large bedload is moved In one instance, suspended sediment after a storm rose from 22 8 p p m at 1 11 cumecs to 1,945 7 p p m at 51 cumecs This material is an important form of nutrient loss from the ecosystem

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Regulation of larval corpora allata in *Galleria mellonella*

IN insects metamorphosis occurs when the corpora allata stop secreting juvenile hormone, but it is not known how the production of juvenile hormone is regulated Wigglesworth[1] first indicated that the nervous connections from the brain to the corpora allata provide a mechanism for control, this control could be nervous or neurohormonal In many adult insects, the corpora allata seem to be inhibited *via* their nervous connections to the brain, since in most cases severing these nerves activates the glands[2,3] Whether the same is true for larvae has not been demonstrated conclusively Activation of the corpora allata by neurosecretion has been demonstrated in many adult insects, for example *Tenebrio molitor*[2], *Leptinotarsa decemlineata*[4] and *Rhodnius prolixus*[5], and the production of an allatotropic hormone by the larval brain has also been suggested[6–8] We have now investigated the control of corpora allata function in last instar larvae of *Galleria mellonella,* encouraged by the discovery that in this species the implantation of brains at the beginning of the last larval instar causes an extra larval moult[9] Our results show that the corpora allata are activated by a neurohumoral factor from the brain during larval development and that they are inactivated by nervous inhibition just before metamorphosis

We first confirmed that the implantation of brains causes supernumerary moults, by implanting brains dissected from 48–120 h last instar larvae under the dorsolateral abdominal epidermis of water-anaesthetised hosts During the first 48 h of the last larval instar, the hosts responded to the implantation of three brains by undergoing a supernumerary moult (Table 1) Identical results were obtained after the implantation of corpora cardiac–corpora allata or brain–cardiac–allata complexes[10,11] The capacity of the host larvae to respond to the implanted brains was lost about 60 h after the last larval–larval ecdysis

Table 1 Development of last instar larvae implanted with three brains at different times after the last larval–larval ecdysis

Hosts Age at time of implantation (h)	No	Larval–larval ecdysis		Larval–larval ecdysis	
		% of insects	Length of instar (d)	% of insects	Length of instar (d)
12	89	55	5 0	45	8 0
48	9	44	6 3	56	7 4
60	11	9	8 0	91	8 5
120	28	0	—	100	8 3

Brains came from donors 48–120 h after the last larval–larval ecdysis

To distinguish whether the implanted brains activate the corpora allata[12,13] or the prothoracic glands, we performed the same implantations on 0–12 h last instar larvae which had been allatectomised The allatectomised larvae did not respond to the implanted brains, indicating that in the previous experiments the brains were primarily stimulating the corpora allata Thus before the last larval instar, the corpora allata appear to be activated by a factor from the brain During the last larval instar, either the brain no longer produces or releases this factor, or the glands are inhibited from responding to it

We investigated the capacity of brains from sixth and seventh (last) instar larvae to induce extra larval moults in sensitive last instar larval hosts (Table 2) The ability of the brain to activate the corpora allata seems to change during development, being most potent during the first part of the instar and declining just before the moult Implanted brains from 120 h last instar larvae were the least able to elicit extra larval moults This corresponds to the period between 54 and 152 h in the last larval instar when the changes from the larval to the pupal programme of development can no longer be affected by large amounts of exogenous juvenile hormone[11,15] Since brains from 0–72 h last instar larvae can induce an extra larval instar when implanted into sensitive hosts, while the corpora allata lose their sensitivity to implanted brains about 48–60 h after the last larval–larval ecdysis, reactivation of the corpora allata *in situ* might be prevented by nervous stimuli We have found that severing the nerves to the glands 72 h after the last larval–larval ecdysis partially restores their activity (Table 3), since two of our experimental animals moulted into supernumerary larvae, whereas no effect was seen in a comparable number of sham-operated animals The length of the instar to the larval-pupal ecdysis in those animals which had the nervous connections severed was prolonged significantly, indicating at least some restoration of activity of the corpora allata Since a definite, but less pronounced, prolongation of the instar was observed in the sham-operated animals, injury apparently contributes to this effect Most significantly, severence of the nerves rendered host larvae sensitive to the implantation of brains, at a time when unoperated larvae did not respond The fact that only 20% of these animals underwent an extra larval moult is probably due to damage caused to the glands during nerve severence We conclude that there is probably a nervous inhibitory influence on the corpora allata which develops between 48 and 60 h after the last larval–larval ecdysis

Table 2 Induction of extra larval–larval ecdyses by implantation of three brains from donor larvae of different ages into 0–12 h last instar larval hosts

Age of donor	No of larvae	% of insects forming supernumerary larvae	% of insects forming pupae
24–36 h, 6th instar	16	94	6
Premoult 6th instar	16	37	63
0–12 h, 7th instar	34	71	29
48 h, 7th instar	23	61	39
72 h, 7th instar	37	44	56
120 h, 7th instar	16	91	81
Moveable prepupa*	17	35	65
Sham operated	25	0	100

* Eye pigment stage 3–14 (ref 14)

Since the implanted brains activate the corpora allata through the haemolymph, the activating factor appears to be of a neurohormonal nature Further evidence for this comes from the observation that the number of supernumerary larvae resulting from brain implantation increased with the number of brains implanted (Fig 1)

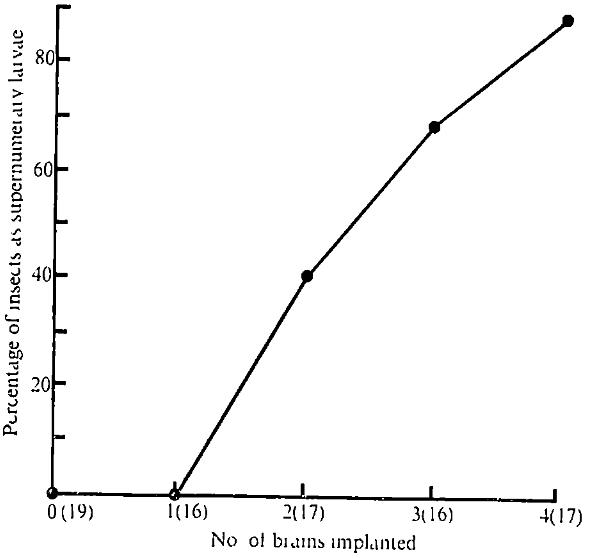

Fig. 1 Extra larval development as a function of the number of brains implanted into larvae 0–12 h after the last larval–larval ecdysis The numbers in parentheses are the numbers of experimental animals

The most prominent neurosecretory cells in the brain of larval *G mellonella* comprise a heterogeneous group found in the pars intercerebralis (ref 16 and our unpublished work) To test whether the activating factor is produced by cells in this region, we destroyed bilateral regions of the brain selectively by microcautery and implanted the cauterised brains into sensitive hosts (Table 4) Significantly fewer extra larval moults were elicited by brains cauterised in the pars intercerebralis This suggests that the allatotropic factor is a neurohormone produced by neurosecretory cells in the pars intercerebralis

Our results show that in larval *G mellonella* the corpora allata are activated by a neurohumoral factor from the brain, possibly a neurohormone produced in the pars intercerebralis A nervous inhibitory influence on gland function probably develops between 48 and 60 h after the last larval-larval ecdysis, subsequently being reinforced by a decrease in the production or release of the allatotropic factor by the brain

Table 3 Development of 72 h last instar larvae after severence of the nerves innervating the corpora cardiaca–corpora allata, and implantation of brains 0–24 h later

Operation	No of larvae	Larval–larval ecdysis		Larval–pupal ecdysis	
		% of insects	Length of instar (d)	% of insects	Length of instar (d)
Host plus three brains	20	0	—	100	7 5±0 8
Nerves severed	31	7	15 0±4 2	93	16 0±7 7
Nerves severed plus three brains	35	20*	16 3±5 5	80	13 2±4 1
Sham operated	29	0	—	100	11 0±3 2

Brains came from donors 48–120 h after the last larval–larval ecdysis

* Three animals were intermediates two were score 3, one was score 4 (ref 11)

The regulatory mechanism for the control of the function of the corpora allata is undoubtedly more complex than the situation we have described Juvenile hormone has been reported to stimulate the secretion of ecdysone by the prothoracic glands[17,18], and the corpora allata appear to be influenced by ecdysone, whose production by the prothoracic glands is under the control of neurohormones

Table 4 Induction of extra larval–larval ecdyses by implantation of brains cauterised in different regions in 0–12 h last instar larvae

Operation	No of larvae	% of insects forming supernumerary larvae	% of insects forming superlarvae
Sham cautery	18	72	28
Cautery area 1	24	25	75
Cautery area 2	19	63	37
Cautery area 3	19	58	42
Cautery area 4	20	50	50
Cautery area 5	20	53	47

Each host was implanted with three brains from 0–12 h last instar larvae Area 1, pars intercerebralis, area 2, corpora pedunculata, area 3, anterior to corpora pedunculata, area 4, lateral to corpora pedunculata, area 5, medial to tritocerebrum

from the brain[18] The brain obviously plays a central role in these relationships, and experiments are under way to investigate the possibility of endocrine feedback mechanisms in the brain

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Cyclic GMP and cyclic AMP levels in normal and transformed fibroblasts

THE 3′–5′ cyclic nucleotides, cyclic AMP and cyclic GMP have been implicated in the control of the growth of cultured fibroblasts in that early, transient decreases in intracellular cyclic AMP[1–3] and increases in cyclic GMP[4] are observed on reinitiation of the growth of quiescent mouse fibroblasts by serum Moreover additions of high, nonphysiological concentrations (10^{-5} M to 10^{-3} M) of either cyclic AMP or cyclic GMP (or their butyrated analogues) either suppress the induction of DNA synthesis after serum addition[5] or initiate substantial increases in DNA synthesis respectively[4] in quiescent mouse fibroblasts Cyclic nucleotide concentrations in asynchronously-growing cell cultures are an average over the entire cell cycle and depend for cyclic AMP on the proportion of cells in a particular phase The average cyclic AMP level is often correlated with growth rate[6], although this may not be such a reliable parameter for cell growth as the relative proportions of G1, S, G2 and M vary with cell type and the culture conditions[7] The larger changes in cyclic GMP are correlated directly with the initiation of fibroblast growth when cells are moving out of the G0 state or by passage through the M and G1 boundary and are not exhibited during the remainder of the cell cycle (W S, and P S R, unpublished) Thus cyclic GMP concentrations and the ratios of cyclic AMP to cyclic GMP may be more sensitive and reliable indicators of the growth state of a cell

Here we demonstrate that nontransformed fibroblasts show a large difference in cyclic GMP and cyclic AMP content and cyclic AMP cyclic GMP ratios for growing and quiescent fibroblasts, no such differences occur in transformed fibroblasts These results suggest fundamental differences in the control of cyclic GMP concentrations between normal and transformed fibroblasts in monolayer cultures

Addition of fresh medium and 20% serum to quiescent cultures of Swiss mouse 3T3-4A fibroblasts caused early, transient increases in cyclic GMP and decreases in cyclic AMP concentrations, reaching maximum and minimum levels after 10 to 20 min, respectively (Table 1*a*), similar to the changes observed in quiescent BALB/c 3T3 cultures[4] The concentration of cyclic nucleotides present in a cell is more accurately expressed relative to the total protein content rather than the DNA content or the number of cells as both the total protein and the volume of a cell change in harmony during the cell cycle[8] Prostaglandin E_1 (PGE_1) elevates intracellular cyclic AMP concentrations in 3T3-4A cultures by a stimulation of adenyl cyclase activity[1] Addition of PGE_1 with the new medium caused increased levels of cyclic AMP, suppressed the rise in cyclic GMP observed after 20 min, and reduced the induction of DNA synthesis by a third An increase in cyclic AMP presumably inhibits the cyclic GMP increase as PGE_1 does not directly affect the membrane-bound guanyl cyclase (P S R, and W S, unpublished)

Table 1 Kinetics of cyclic nucleotide changes on reinitiation of cell growth

Time (min)	Cyclic AMP	Cyclic GMP	Cyclic AMP/ cyclic GMP
a Serum addition to 3T3			
0	19	0 7	28
5	2 0	3 5	0 6
10 (PGE_1)*	2 4 (4 4)*	3 6 (0 5)*	0 5 (8 4)*
20 (PGE_1)*	3 1 (26)*	2 2 (0 5)*	1 4 (48)*
60 (PGE_1)*	5 8 (9 5)*	2 7 (2 2)*	2 1 (4 3)*
b Leucine addition to SV3T3			
0	10 0	3 8	2 6
5	7 2	3 2	2 2
10	7 6	3 4	2 3
20	7 6	4 9	1 5
60	8 8	3 6	2 5

a, Swiss mouse 3T3-4A cultures were grown in 9-cm Petri dishes for 10 d (2 5 × 10^6 cells per dish)[17], the medium was then replaced with fresh DEM (ref 17) and 20% serum with* or without 10 μg ml^{-1} prostaglandin E_1 (PGE_1) and cultures were isolated at the stated times for cylic nucleotide determinations[4 19] Parallel cultures were also radioactively labelled with ^{3}H–thymidine from 18–22 h or from 8–32 h after medium change for DNA synthesis and autoradiography respectively[4] The c p m incorporated into DNA per 10^6 cells (% of cells with radioactively labelled nuclei) were 3 4 × 10^3 (1 2%), 200 × 10^3 (91%), and 90 × 10^3 (36%) for the original cultures, cultures with fresh medium, and cultures with fresh medium and PGE_1 respectively *b*, Simian virus 40 transformed 3T3-4A cells (SV3T3) were grown to a density of 6 1 × 10^6 per dish, cell monolayers were washed twice with DEM lacking leucine, and DEM containing 1/200 of the normal leucine concentration and 5% dialysed serum was added back and incubated a further 2 d (5 5 × 10^6 per dish)[12] Then regular concentrations of leucine were restored and cultures were isolated as above Cell density 30 h later was 9 9 × 10^6 per dish, DNA synthesis and cell cycle data are shown in Table 3 Half of each sample for cyclic nucleotide determination was digested with 3′–5′ cyclic nucleotide phosphodiesterase and the values obtained (0 2–0 3 pmol for cyclic AMP and 0 6–0 9 pmol for cyclic GMP) were deducted to yield the final results[4,14] Duplicates agreed to within 15% and results are expressed in pmol of cyclic nucleotide per mg of cell protein[20]

Table 2 Cyclic nucleotide concentrations in growing, resting, and serum-activated cultures

Cell type	Growth state	Labelled nuclei (%)	Cyclic AMP	Cyclic GMP	Cyclic AMP/cyclic GMP
BALB/c 3T3 mouse (10% serum)	Growing	93	8.4	2.2	3.4
	Resting	0.4	15.4	0.8	19
	Activated	86	4.7	4.1	1.1
BALB/c 3T3 mouse (2% serum)	Growing	74	6.8	2.3	3.0
	Resting	0.5	9.9	0.7	14
	Activated	88	2.4	9.8	0.25
3T3-4A Swiss mouse	Growing	73	7.2	2.9	2.5
	Resting	0.5	16.1	0.6	29
	Activated	91	3.2	4.2	0.78
Secondary mouse embryos	Growing	79	4.5	2.3	2.0
	Resting	1.4	17.4	1.1	16
	Activated	71	3.3	10.0	0.33
BSC-1 monkey	Growing	93	7.2	2.1	3.3
	Resting	0.5	13.3	0.7	18
	Activated	95	3.4	4.4	0.77
BHK hamster	Growing	56	7.6	3.5	2.1
	Resting	2.3	13.2	1.6	8.4
	Activated	86	2.9	15.4	0.19
3T6 Swiss mouse	Growing	86	6.5	2.0	3.3
	Resting	10	9.7	0.8	13
	Activated	92	2.5	2.9	0.84
SV3T3 transformed mouse	Growing	92	8.5	4.9	1.7
	'Resting' (confluent)	84	8.6	4.5	1.9
	Activated	97	7.0	4.3	1.6
PyBHK transformed hamster	Growing	80	5.6	4.7	1.2
	'Resting' (confluent)	83	7.4	4.3	1.7
	Activated	89	6.2	4.5	1.4

Twenty Petri dish cultures[17] for cyclic nucleotide determinations were isolated after 2 or 3 d on reaching a quarter to half of the final cell density (growing), after 8–9 d (resting) (3–4 d after the cessation of DNA synthesis in nontransformed cells or at confluency for the transformed cells), or after 20 min following addition of fresh DEM and 20% serum to 'resting' cultures (activated) (Table 1). The cyclic nucleotide amounts are expressed in pmol per mg cell protein and DNA synthesis by the percentage of cells with radioactively labelled nuclei after exposure to ^{3}H–thymidine for 24 h before isolation of growing cultures or from 8–32 h after the time of medium change for resting and activated cultures. Serum concentrations and the cell densities upon isolation ($\times 10^6$) of growing and 'resting' (or activated) cultures respectively were for BALB/c 3T3 in 10% serum 0.85 and 2.2, in 2% serum 0.65 and 1.1 (serum growth activated cultures isolated after 10 min instead of 20 min), for Swiss mouse 3T3-4A in 10% serum 1.6 and 2.8, for secondary mouse embryo fibroblasts in 10% calf serum or 0.5% horse serum 2.5 and 6.4, for African green monkey kidney fibroblasts (BSC–1) in 10% serum 0.61 and 4.0, for baby hamster kidney fibroblasts (BHK) in 10% and 1% serum, 2.9 and 0.36 (confluency about 10). for Swiss mouse 3T6 fibroblasts 7.2 and 18.0, for simian virus 40 transformed 3T3-4A (SV3T3) in 10% serum, 2.6 and 12.8 (confluency), and for polyoma virus transformed BHK (PyBHK) in 10% serum, 8 and 25.2 (confluency).

The cyclic nucleotide contents of different fibroblasts were compared when cultures were randomly growing, in a quiescent state mainly as a consequence of the exhaustion of growth factors in serum[9] or in a synchronised growing state 20 min after fresh medium and 20% serum were added to quiescent cultures (Table 2). When growing cells ceased to divide and became quiescent, cyclic AMP levels increased by about two-fold (fourfold for mouse embryos) while cyclic GMP levels decreased by a factor of about two to three. On the initiation of growth of the quiescent cultures, cyclic AMP levels fell three- to fivefold while cyclic GMP rose four- to twelvefold. Because of the parallel but reciprocal changes in cyclic GMP concentrations the cyclic AMP:cyclic GMP ratio reflected these changes more dramatically. It was lower for growing cells (2 to 4), high when cells became quiescent (12 to 30) either when in contact (BALB/c 3T3 in 10% serum) or in sparse cultures (BALB/c 3T3 with 2% serum), and about unity or below in the synchronised growth state (activated for 20 min). Baby hamster kidney (BHK) and mouse 3T6 fibroblasts which contained a significant fraction (2.3% and 10%) of cells synthesising DNA also have the lowest cyclic AMP:cyclic GMP ratios of the 'resting' fibroblasts tested. In contrast, the cyclic nucleotide concentrations for virally transformed fibroblasts SV3T3 and PyBHK did not change significantly between the three growth states (Table 2) and cyclic GMP levels were a factor of two higher than for the growing fibroblasts. Since the transformed cells failed to exhibit density-dependent inhibition of growth[9,10], they were actively synthesising DNA even when the cultures were confluent (Table 2).

Artificially depriving the culture medium of required nutrients is another reversible method of controlling the growth of both nontransformed[11] and transformed[12] fibroblasts in culture. Depending on the depleted nutrient the 3T3-4A fibroblasts cease to grow in the G1 phase or at random, whereas the transformed fibroblasts, SV3T3, only stop at random in the cell cycle. Restoration of the depleted nutrients initiates growth and cell division. Only the 3T3-4A fibroblasts that were synchronously arrested in the G1 phase of the cell cycle by deprivation of histidine and glutamine showed significant increases or decreases in cyclic GMP and cyclic AMP:cyclic GMP ratios, respectively, 20 min after addition of the depleted amino acids (Table 3). The kinetics of the changes were similar to those shown in Table 1 on growth initiation by serum (not shown). Methionine deprivation arrested cells at random in the cell

Table 3 Reinitiation of growth in amino acid-starved cultures

Culture	Labelled nuclei (%)	Cyclic AMP	Cyclic GMP	Cyclic AMP/cyclic GMP
3T3−Met	4	7.3	2.5	2.8
3T3+Met	21	7.7	4.0	2.0
3T3−His−Glu	3	10.0	0.8	13
3T3+His+Glu	86	6.5	12.5	0.5
SV3T3−Leu	1	10.0	3.8	2.6
SV3T3+Leu	67	7.6	4.9	1.6

Swiss 3T3-4A (3T3) and SV3T3 cells were grown for 3 d in complete media (Table 1) (1.0 and 6.1 $\times 10^6$ cells per dish), and then in medium containing 1/100 of the normal concentration of methionine (Met) or histidine and glutamine (His, Glu) for 3T3 (ref. 11) or 1/200 of leucine (Leu) for SV3T3 (ref. 12) (Table 1). After 3 d for 3T3 (1.8 and 1.0 $\times 10^6$ cells for minus Met or His and Glu) or 2 d for SV3T3 (5.5 $\times 10^6$ cells), half the cultures were supplemented with the normal concentrations of the depleted amino acids and nine or ten petri dish cultures were isolated from both the original starved cultures (−) and those cultures reinforced for 20 min with the added ingredients (+). The pmol cyclic nucleotides per mg cell protein and the % of cells with radioactively labelled nuclei after exposure to ^{3}H–thymidine from 8–32 h after additions were recorded as in Table 1. Cell cycle results from the average DNA content per cell measured in a microfluorometer[11] expressed as the percentage of cells in G1, S, G2 were for 3T3–Met 71, 12, 17, for 3T3–His–Glu 94, 0, 6, for growing 3T3–4A (Table 2) 57, 26, 17, for resting 3T3–4A (Table 2) 94, 0, 6, for SV3T3-Leu 38, 29, 23, for growing SV3T3 (Table 2) 45, 30, 25 (ref. 12).

cycle and no comparable changes in cyclic nucleotide concentrations were observed when it was restored (Table 3) Leucine deprivation arrested SV3T3 cells randomly throughout the entire cell cycle (Table 3) and no significant early increases in cyclic GMP levels were observed when growth was initiated by restoration of this amino acid, though cyclic AMP levels fell by about 25% after 20 min (Table 1*b*)

Addition of purified growth-initiating molecules, phytohaemagglutinin or fibroblast growth factor from bovine pituitary glands to quiescent cultures of human peripheral blood lymphocytes[13] or BALB/c 3T3 mouse fibroblasts[14], respectively, caused large early increases in intracellular cyclic GMP but little change in cyclic AMP Conversely addition of PGE$_1$, which raised intracellular cyclic AMP, reversed the induction of DNA synthesis in quiescent 3T3-4 mouse fibroblasts and stopped growth in dividing cultures[1,2] That both fibroblast growth factor and PGE$_1$ stimulated membrane-bound guanyl[14] or adenyl cyclase[1] enzymes, respectively, suggest that cyclic GMP could act as a positive while cyclic AMP acted as a negative intracellular signal for controlling the growth of cultured cells We have shown here that cyclic GMP concentrations as well as those of cyclic AMP (refs 1 and 2) are closely correlated with the growth state of different cultured fibroblasts and that parallel but reciprocal changes are observed in the cyclic nucleotide concentrations in transition between these growth states[15]

The changes in cyclic nucleotide concentrations, particularly those of cyclic GMP, are largely lost when the cells become transformed, whereas the average cyclic GMP concentrations were approximately twice as high in transformed as in nontransformed fibroblasts The failure to observe changes in cyclic GMP for transformed cells probably reflects the failure to arrest the transformed cells in the G1 or G0 phase of the cell cycle Whether this is caused by a lesion in the production of the putative pleiotypic programme of the cell[16,17] or as a consequence of the failure of fibroblastic factors from delivering their growth controlling signals[18] is still uncertain at present

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Inhibition of cell growth by oxygenated derivatives of cholesterol

CELLS growing in a chemically defined, sterol-free medium must synthesise cholesterol or, in the case of L cells, desmosterol[1] for membrane formation Studies by others showed that when cholesterol or desmosterol was added to L-cell cultures the exogenous sterol was utilised and the rate of sterol synthesis was diminished, presumably by a negative feedback mechanism[2,3] Our observations[4], however, provide new information regarding the structures of sterols that affect sterol synthesis Exogenous cholesterol and various metabolically related steroids do not inhibit sterol synthesis in mouse liver cell or fibroblast cultures under conditions where derivatives of cholesterol oxygenated in the 7, 20, 22 or 25 positions are highly inhibitory These inhibitory sterols, at concentrations of 0 02–0 2 μg ml^{-1} specifically depress the activity of the regulatory enzyme in the sterol synthetic pathway, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (EC 1 1 1 34), within 6 h

We now report that prolonged incubation of L cells with the inhibitory steroids diminishes the concentration of cellular desmosterol, results in the reduction or cessation of the growth of the culture, and creates a requirement for an external supply of an appropriate sterol or for a sterol precursor further along the biosynthetic pathway than HMG-CoA

L cells were grown in chemically defined medium[4] in Falcon culture flasks (25 cm^2) for 1–3 d before the introduction of the experimental media as described below The sources of the sterols and procedures used to purify them have been reported previously[4] Steroids suspended in a solution of 5% crystallised bovine albumin were added to cultures previously refed with fresh medium so that the final concentration of albumin was 0 05% or 0 1% Control cultures were fed medium containing an equivalent amount of albumin The cultures were incubated on a rotating table (20 r p m) at 37° C and the medium was renewed every 3 d At the end of the incubation period cells adhering to the flask were washed twice with physiological saline, digested with 0 1 N KOH and assayed for protein[5,6] The amount of cellular protein in a culture is a good measure of cell growth[6,7], at least during the log phase of L-cell culture[8] Our preliminary studies showed that there was a good correlation between cell number and the amount of protein or DNA in growing L-cell cultures and in cell cultures incubated with different steroids as described below

The most potent inhibitor of cell culture growth was 25-hydroxycholesterol followed, in order of decreasing activity, by 20α-hydroxycholesterol, 7-ketocholesterol, and 7α-hydroxycholesterol (Fig 1*a*) The first three sterols inhibited growth of cultures by more than 90% at 1, 2 5 and 10 μg ml^{-1}, respectively In contrast to results obtained

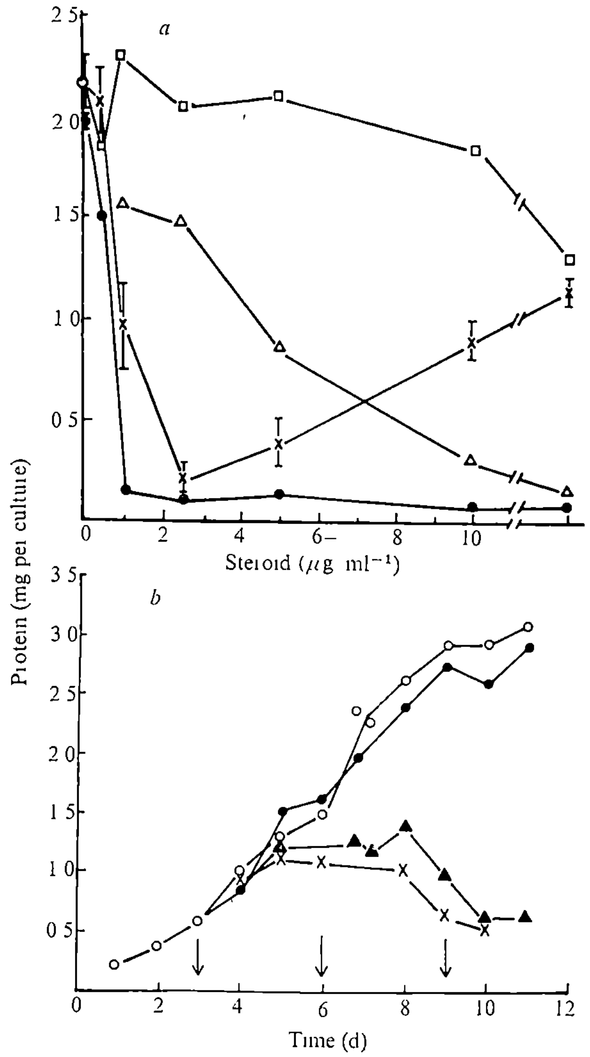

Fig 1 *a*, Inhibition of cell growth by some oxygenated derivatives of cholesterol Cultures were grown for 2 d in normal medium then in medium containing indicated concentrations of 25-hydroxycholesterol (●), 20α-hydroxycholesterol (×), 7-keto-cholesterol (△), or 7α-hydroxycholesterol (□) Cultures were refed with experimental or control media after a further 3 d and cellular protein was analysed 2 d after refeeding Vertical lines represent range of values for at least 3 replicate flasks *b*, Effects of 20α-hydroxycholesterol alone or in combination with acetate or mevalonate on the time course of cell growth Cultures were grown for 3 d in normal medium then (first arrow) in control medium (○) or in medium containing 20α-hydroxycholesterol, 10 μg ml^{-1} (▲), 20α-hydroxycholesterol, 10 μg ml^{-1} and MVA, 1 mg ml^{-1} (●), or 20 α-hydroxycholesterol, 10 μg ml^{-1} and acetate, 1 mg ml^{-1} (×) The cultures were refed with control or experimental media on days indicated by the second and third arrows

with the other inhibitory steroids, cell growth improved when 20α-hydroxycholesterol was increased beyond that required for maximum inhibition, possibly due to the utilisation of this diol to meet the sterol requirement for cell growth Under similar conditions, 7α-hydroxycholesterol at 10 μg ml^{-1} was not inhibitory but at 50 μg ml^{-1} did inhibit growth by 50% In experiments not shown, 7β-hydroxycholesterol was more inhibitory than 7α-hydroxy-cholesterol

The relative effectiveness with which these sterols inhibited the growth of cultures correlated well with their previously reported capacities to inhibit sterol biosynthesis and to depress levels of HMG-CoA reductase activity[4] Sterol concentrations required to arrest growth within 3 or 4 d, however, were several times higher than those necessary to inhibit sterol synthesis within 6 h The apparent discrepancy between these values may indicate that sterol synthesis must be almost totally blocked to obtain the marked depression in the growth of the cultures that we observed The extent to which cell growth could be adversely affected by an inhibitory sterol also depended on the stage of growth of the culture A growing, sparse culture was far more susceptible to inhibition than a nearly confluent, dense culture

Figure 1*b* illustrates the time course of the inhibitory effect of 20α-hydroxycholesterol on the growth of cultures The amount of protein in the cultures increased for the first 2 d after the addition of the sterol, remained essentially constant for about 3 d, then declined Supplementation of the medium with mevalonate (MVA), the product of the reaction catalysed by HMG-CoA reductase, counteracted the effect of 20α-hydroxycholesterol and resulted in a normal rate of growth In contrast, supplementation with acetate did not relieve the inhibitory effects of the sterol

In other experiments the inhibitory effect of 20α-hydroxy-cholesterol, 25-hydroxycholesterol or 7-ketocholesterol was abolished by the addition of desmosterol or MVA Concentrations of desmosterol and MVA required to fully counteract inhibition were about 25 μg ml^{-1} and 1 mg ml^{-1}, respectively Addition of MVA or desmosterol alone to control cultures did not appreciably alter the cell growth, suggesting that, under usual culture conditions, the rate of growth was not limited by the rate of sterol synthesis The abilities of various steroids and their precursors to counteract the inhibitory effect of a 20α-hydroxycholesterol on the culture growth are compared in Table 1 Cholesterol was nearly as effective as desmosterol, whereas cholestanol, Δ^7-cholestenol, cholest-4-en-3-one and cholest-5-en-3-one were moderately effective Other steroids, HMG, or acetate, showed little or no ability to counteract inhibition by 20α-hydroxycholesterol

Estimates of cellular desmosterol after incubation with the inhibitory sterols alone or in combination with the various additions to the medium shown in Table 1 were made after hydrolysing the washed cells with 1 N KOH in 50% ethanol for 2 h and extracting the neutral fraction with ether Sterols in the extracts were analysed by gas chromatography as the free sterols or after conversion to the trimethylsilyl ethers[4,9] After 5 d of incubation with the inhibitory sterols in amounts sufficient to inhibit cell growth by 70% or more, desmosterol concentration in the surviving cells was less than 1 μg mg^{-1} protein, markedly reduced from that of the control cells (11 4±4 μg mg^{-1} protein) When the growth inhibitory effects of 20α-hydroxy-cholesterol, or 25-hydroxycholesterol were counteracted by the addition to the medium of a non-inhibitory sterol, concentrations of desmosterol in the cells remained low

Table 1 Effect of sterols and their precursors on the growth of cells incubated with 20α-hydroxycholesterol

Additional compounds	Cellular proteins (% of the control)
None	30 ± 4 (4)*
Desmosterol	95 ± 3 (4)
MVA (sodium salt)	92 ± 1 (4)
Cholesterol	77 ± 10 (5)
5α-Cholestanol	68
Δ^7-Cholestenol	67
Cholest-4-en-3-one	57
Cholest-5-en-3-one	49
5α-Cholestan-3-one	36
β-Sitosterol	35
HMG (sodium salt)	17 ± 2 (3)
Acetate (sodium salt)	17 ± 1 (3)

All flasks except controls were incubated for 5 d with medium containing 20α-hydroxycholesterol (10 μg ml^{-1}) and additional compounds as indicated (steroids, 50 μg ml^{-1} MVA, HMG, and acetate, 1 mg ml^{-1})

*Mean ± s e Numbers in parentheses indicate number of experiments

If, however, the medium contained mevalonate instead of a non-inhibitory sterol, desmosterol increased to 12.8±2.6 μg mg^{-1} protein, and several unidentified sterol peaks were also present on the chromatogram. Small amounts of the inhibitory sterols and larger amounts of the non-inhibitory sterols administered with the inhibitors were detected in the cell extracts.

The results of these experiments demonstrated that L cells became severely deficient in desmosterol when incubated with certain oxygenated derivatives of cholesterol which specifically inhibit sterol biosynthesis at the level of HMG-CoA reductase. The growth of the cultures was then greatly depressed unless an appropriate sterol or sterol precursor was supplied exogenously. Several explanations of the effect of the inhibitory sterols on culture growth can be brought forward. Possibly the inhibitory sterols compete with desmosterol for sites in the membrane with resulting effects on membrane function. Specific repression of HMG-CoA reductase activity by the inhibitors would then be a coincidental and unrelated effect. We believe that it is more likely that the effects of the inhibitors on cell growth stem from repression of the enzyme activity and the subsequent restriction of sterol synthesis. Partial depletion of cholesterol from erythrocytes and mycoplasma results in increased membrane fragility[10,11], so that the growth retardation of L-cell cultures that were unable to synthesise sterols may have been due to membrane instability and cell death. On the other hand, it is now apparent that altering the sterol concentration of artificial cell membranes, and membranes of erythrocytes or mycoplasma changes the fluidity of the membrane, the rates at which various substances are transported through the membrane, and the activities of membrane-localised enzymes[12-14]. Thus, the inhibitory sterols may influence growth of the culture in ways other than by increasing membrane fragility.

For the first time the amount and kind of sterol in living mammalian cells can be manipulated through the use of specific inhibitors of sterol synthesis so that it should be possible to investigate in detail the role of sterols in membrane structure and function and their relationship to cell growth.

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Morphological and functional maturation of human thymic epithelium in culture

ORGAN culture of embryonic avian and murine thymus[1-5], as well as explants of guinea pig and rabbit thymus[6-10], have been observed to produce outgrowths which in some cases were described as predominantly lymphoid in nature, epithelial in other cases. Two publications have described human embryonic thymus in culture, using the plasma clot technique[9,10]. We have established epithelial outgrowths from explants of human thymic tissue and maintained these cultures for periods up to 3 months.

Fresh thymic tissue was obtained at surgery from 20 children, ranging in age from 1 week to 13 yr, with various surgically correctable cardiac lesions. Capsular tissue and haemorrhagic areas were dissected away and the remaining tissue was finely minced in Dulbecco's modification of Eagle's medium, containing 100 units ml^{-1} of penicillin, 100 μg ml^{-1} of streptomycin, and 2.5 μg ml^{-1} of amphotericin-B, supplemented with 30% foetal bovine serum (FBS, Flow Laboratories). The preparation

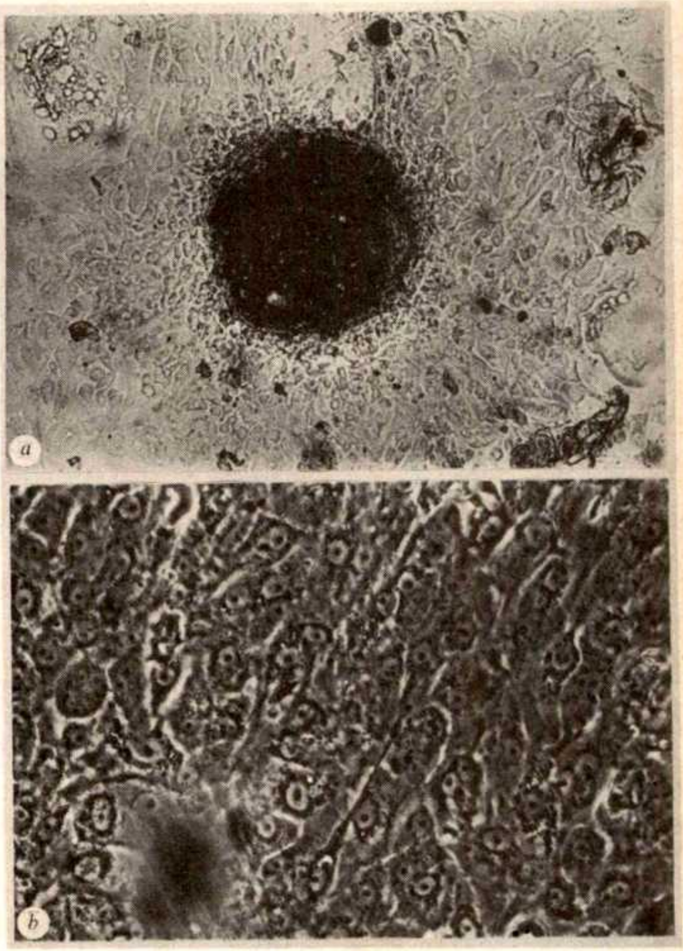

Fig. 1 Living cultures of human thymic epithelium at 30 d. *a*, The primary explant is present as a dense central mass with a surrounding monolayer of large epithelial cells in a mosaic-like arrangement (× 26); *b*, phase contrast shows the cells to have a large central nucleus with a prominent central nucleolus and marked cytoplasmic granularity (× 104).

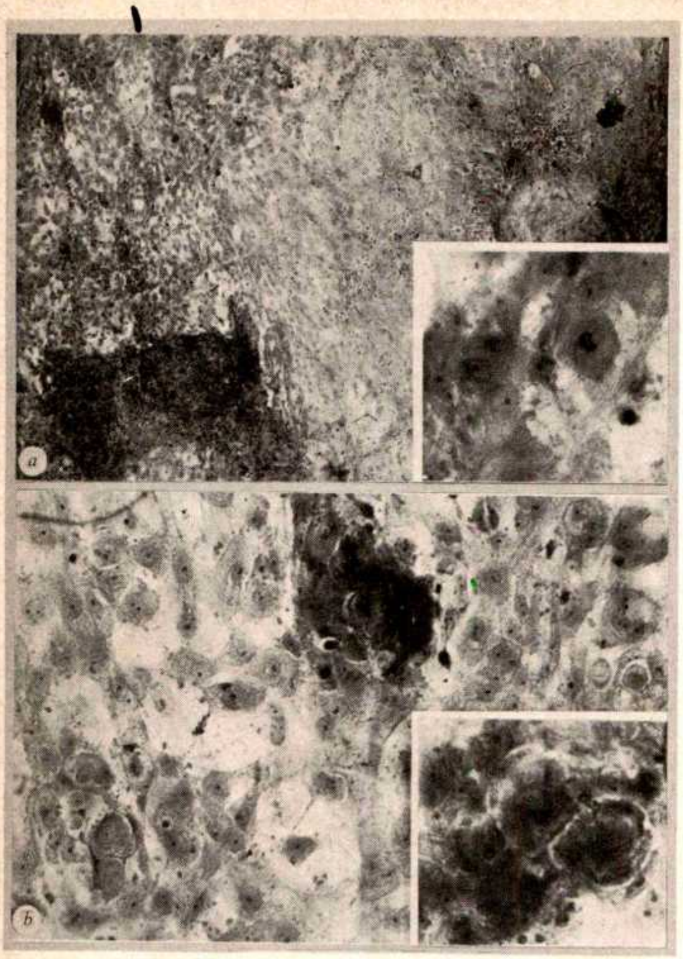

Fig. 2 Ten day culture of human thymus on glass coverslip, fixed *in situ* and stained with haematoxylin and eosin. *a*, Two primary explants are present as dense masses of dark-staining cells, with the epithelial outgrowth spreading between the two explants as sheets of large cells with abundant, somewhat granular cytoplasm (× 3.76). Inset, mitotic figure at the periphery of advancing epithelial growth (× 60.16); *b*, cell aggregates resembling Hassal's corpuscles in various stages of development; left lower corner: a group of cells showing early cyst formation with cell crescenting (× 37.60). Inset, dense aggregate of cells arranged in concentric patterns around central amorphous eosinophilic material (× 60.16).

was then washed with a threefold volume of the same medium, allowed to settle for 5 min at room temperature, and the resultant cell suspension removed by pipette. The tissue fragments remaining were washed three times by this method, then transferred to culture. In most cases the fragments, in a small volume of medium sufficient only to cover the culture surface, were added to plastic tissue culture flasks (Falcon number 3012 and 3024). In several cases, fragments were also cultured in plastic tissue culture dishes (Falcon number 3005) with and without 22 mm glass coverslips, to be subsequently fixed and stained for cell morphology. Incubation was at 37° C in a humidified atmosphere containing 5% CO_2. On day 3, an equal volume of fresh medium was added.

By days 5–7 an epithelial outgrowth, twice the diameter of the explant, was observed. The medium was replaced twice weekly until a confluent monolayer was obtained, when the FBS concentration in the medium was reduced to 20%. Medium collected from the cultures (conditioned medium) was centrifuged at 550*g* for 10 min at 10° C and the supernatant removed and stored at −20° C. In all flasks an outgrowth of large epithelial cells from the explants was seen (Fig. 1) which produced a confluent monolayer, usually by day 14. Cells morphologically identified as fibroblasts were present in all cultures and persisted in most cases as a 5–10% contamination of the monolayer.

Explants grown on coverslips were fixed *in situ* and stained with haematoxylin and eosin. Aggregates of cells arranged concentrically around central amorphous eosinophilic material were seen scattered singly and in groups in the epithelial outgrowth, both adjacent to and remote from the primary explanted tissue (Fig. 2). These resembled Hassal's corpuscles as seen in sections of normal thymic tissue. Cultures fixed and embedded *in situ* in plastic flasks were examined with the electron microscope and showed desmosome formation between cells, confirming their epithelial nature.

Decrease in the concentration of FBS in the medium below 30% at the time of initial explantation resulted in a proportionate decrease in the rate of epithelial growth but had no effect on fibroblast growth. Consequently, cultures with 20% FBS were overgrown by fibroblasts within 30 d, while those with 10% FBS were overgrown in 14–20 d. When single cell suspensions were cultured in the same conditions, fibroblast growth predominanted. Thymic lymphocytes showed a gradual decrease in number with time in culture, as shown by Wright-Giemsa staining and E rosette formation (a marker of T lymphocytes)[11] of cells shed into the medium from the monolayers, until none was detectable by these means at 30 d in culture.

Conditioned medium collected from the cultures and stored frozen was examined for its ability to influence the capacity of human marrow cells to form rosettes with sheep erythrocytes at 4° C (E rosettes). Marrow obtained by aspiration was separated on Isopaque-Ficoll gradients[11] to remove erythrocytes and enrich for lymphoid cells. Cells from the gradient interface were washed three times with phosphate-buffered saline. Some of the cells were resuspended in PBS to a concentration of 3×10^6 nucleated cells ml^{-1} and assessed for rosette formation after 2 h at 4° C (ref. 11). Rosettes were also enumerated by counting the remaining cells after incubation for 180 min at 37° C with fresh medium, conditioned medium from thymus cultures, and conditioned medium from Chang human liver cells (Flow Laboratores). The results (Table 1) suggest that conditioned media from confluent human thymic epithelial monolayers only were capable of increasing the capacity of unfractionated human marrow cells to form rosettes with sheep erythrocytes in the cold, while non-lympoid cells (Chang human liver), peripheral blood lymphocytes, tonsillar lymphocytes, and cells from an established line of B lymphocytes were unaffected by the conditioned media. The most effective thymic conditioned media were obtained when thymic lymphocytes were no longer present in the cultures.

Table 1 Percentage of E rosettes

Test media	Marrow A	Marrow B	Marrow C	Cell preparation: Peripheral blood lymphocytes	Tonsillar lymphocytes	Cultured human lymphocytes	Cultured human liver (Chang)
Saline	5	17	14	50	29	0	0
Medium	10	12	12	37	21	0	0
Chang	13	13	13	49	24	0	0
Thymus	22	33	32	46	27	0	0

Bone marrow cells from three donors (A, B, and C) were separated on Isopaque-Ficoll gradients, washed three times with phosphate-buffered saline (PBS) and adjusted to 3×10^6 nucleated cells ml^{-1}. A portion of cells were resuspended in PBS and examined for rosette formation with sheep erythrocytes at 4° C (E rosettes). Other portions were resuspended in 1 ml of test medium: fresh medium containing 20% FCS; Chang: conditioned medium from confluent monolayers of Chang human liver cells, stored at −20° C; thymus: conditioned medium from various confluent monolayers of human thymus epithelium, stored at −20° C), incubated at 37° C for 3 h, washed twice with PBS, resuspended in PBS to 3×10^6 nucleated cells ml^{-1}, and examined for formation of E rosettes. Rosettes were evaluated after 2 h at 4° C, and are expressed as a % of nucleated cells present. The capacity for formation of E rosettes of the control cells was unaffected by the thymus culture conditioned media, but the marrow cells showed an increase in E rosette formation with these conditioned media.

A differentiation process within the thymus of lymphocytes originating from stem cells in haematopoietic tissues is central to the concept of a distinct thymus-derived or T-cell population of circulating lymphocytes[12] Evidence for cell-free thymic differentiation factors has been based on reconstitution *in vitro* and *in vivo* of several immune functions and T-cell associated markers in thymectomised and nude mice, using extracts of fresh bovine and murine thymic tissue[13-17] The epithelial stroma of the thymus has been suggested as the source of such differentiation factors[18] Our observations suggest that an epithelium of apparent thymic nature may be obtained *in vitro* from human tissues and that this epithelium may be a source of a factor or factors with biological activity similar to those extracted from fresh bovine and murine thymus

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Asbestos induces selective release of lysosomal enzymes from mononuclear phagocytes

THE association of asbestos inhalation and lung disease has been recognised for many years[1] Asbestosis is a chronic inflammatory reaction in the terminal bronchioles and alveoli of the lung with considerable fibrosis leading to distortion and eventual obliteration of the alveoli There is also an association between asbestosis and the development of bronchogenic cancer[2] and Wagner *et al*[3] have noted the occurrence of diffuse pleural and peritoneal mesotheliomas in individuals occupationally exposed to asbestos dust and in experimental animals following intrapleural injection of asbestos

It as accepted that the initial event after inhalation of toxic particules such as asbestos is their attachment to and often phagocytosis by mononuclear phagocytes (MP) in the pulmonary alveoli[4] As we consider that MP play a central role in the generation of chronic inflammatory lesions[5], we have analysed the interaction of this type of cell with asbestos *in vitro* Previous studies have illustrated the phagocytosis of asbestos by MP (refs 4 and 6) while biochemical evidence of the cytotoxic effects of relatively high doses of asbestos on MP has been provided by Miller and Harington[7] We now show that asbestos, in common with several other substances such as dental plaque[8], immune complexes[9], streptococcal cell walls[10,11] and carrageenan[12]—all of which cause chronic inflammation *in vivo*—induces a rapid, massive and selective release of lysosomal enzymes from MP The release occurs in the absence of any detectable cell death and is accompanied by considerable increases in cellular levels of nonlysosomal enzymes

Our studies have been carried out with cultures of mouse peritoneal MP which are derived from the same bone marrow precursors as the majority of alveolar macrophages[13,14] and are obtainable in sufficient quantities for detailed biochemical investigation MP exposed to asbestos (UICC chrysotile A, 1–50 μg ml^{-1}) show characteristic morphological changes The cells phagocytose asbestos and an increase in the number of cytoplasmic vacuoles, probably secondary lysosomes, is seen At higher doses (25–50 μg ml^{-1}) the cells tend to form clusters and some rounding is seen although the majority of cells retain the cytoplasmic extensions characteristic of normal MP

Measurement of the total level of lysosomal enzymes in MP cultures after exposure to asbestos for 24 h shows that in general there are no significant changes (Table 1) There is, however, a marked redistribution of lysosomal enzyme activity between cells and culture medium (Table 1), significant increases in the proportions of enzymes in the culture medium being seen with concentrations of asbestos as low as 1 μg ml^{-1} This release is directly proportional to the concentration up to 50 μg ml^{-1} The absolute amount of enzyme activity in the culture medium also increases in a dose-dependent fashion

The selective nature of the lysosomal enzyme release is illustrated in Fig 1 Asbestos at concentrations up to 50 μg ml^{-1} causes no significant increase in the amount of

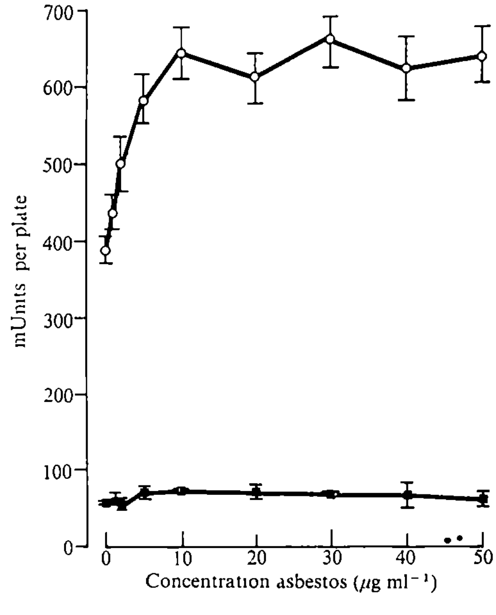

Fig. 1 Lactate dehydrogenase levels in MP culture exposed to increasing concentrations of asbestos for 24 h ○, Activity in cells, ■, Activity in the culture medium Each value represents the mean ± standard deviation of four observations Experimental conditions were as described in Table 1 Lactate dehydrogenase was determined on a Technicon Autoanalyser by the method of Morgenstern *et al*[18]

Table 1 Lysosomal enzyme levels and distribution between cells and culture medium in MP cultures exposed to various concentrations of asbestos for 24 h

Asbestos concentration ($\mu g\ ml^{-1}$)	N–Acetyl–β–D–glucosaminidase nmol product per plate h^{-1}		β–Glucuronidase nmol product per plate h^{-1}		β–Galactosidase nmol product per plate h^{-1}	
	Total	% in medium	Total	% in medium	Total	% in medium
0	3,328±228	13 9±0 4	161 8±4 3	18 2±2 6	216 0±17 3	11 3±0 5
1	3,518±170	20 2±0 7†	171 1±7 4	25 0±4 1	210 7±13 8	15 1±0 9†
2	3,934±387	20 1±0 8†	183 1±22 5	23 2±2 6	221 7±19 3	15 1±0 5†
5	3,782±294	28 5±1 7†	167 9±5 8	33 3±2 7†	207 0±12 8	21 6±1 1†
10	4,150±282*	30 8±1 6†	203 4±10 8†	38 8±2 7†	224 9±14 8	27 4±1 3†
20	3,550±210	43 5±3 4†	180 5±12 6	52 3±4 4†	206 8±11 3	44 5±5 3†
40	3,089±249	53 4±6 3†	167 0±11 3	67 1±4 9†	180 7±14 8	60 9±2 0†
50	3,615±276	61 3±5 9†	183 0±19 2	77 5±5 0†	206 0±8 3	69 8±5 2†

Peritoneal exudates were collected from outbred Swiss mice of either sex 3 d after intraperitoneal injection of 2 ml of proteose-peptone cell suspensions (5 ml) adjusted to a count of 0 8 × $10^6\ ml^{-1}$ were plated in 48-mm Petri dishes (Nunc Jobling Laboratories Division, Stone, Staffs, UK) After culture for approximately 1 h at 37° C in a 5% CO_2 in air atmosphere the medium containing non-adherent cells was removed and the remaining cell sheet washed four times with 4 ml aliquots of phosphate-buffered saline The cells were then cultured overnight in M199 containing 10% (heat-inactivated at 56° C for 30 min) swine serum (Bio-cult Laboratories Ltd , Glasgow, Scotland), Chrysotile asbestos (UICC standard) was sonicated (MSE ultrasonic disintegrator) in serum-free M199 for 2 min M199 and swine serum was then added to give the desired concentration of asbestos and a final swine serum concentration of 10% Asbestos was preincubated in medium overnight Before adding asbestos the cultures were washed twice with phosphate-buffered saline After exposure to asbestos for 24 h the cultures were assayed for various enzymes After removal of the culture medium the cells were removed in 0 1% TX-100 in 0 9% w/v sodium chloride containing 0 1% bovine serum albumin The samples were assayed for N–acetyl–β–D–glucosaminidase[15], β–gluroronidase[16] and β–galactosidase[17] activity by spectrophotometry on a Gilford N–300 spectrophotometer Each value represents the mean ± standard deviation of four observations

* $P < 0\ 05$

† $P < 0\ 01$.

lactate dehydrogenase detected in the culture medium Moreover, even small amounts of asbestos induce highly significant increases in cellular levels of this enzyme (Fig 1, $P > 0\ 01$ at 2 $\mu g\ ml^{-1}$ asbestos) This increase continues in a dose-dependent manner up to 10 $\mu g\ ml^{-1}$ asbestos, above which lactate dehydrogenase activity remains at a constant elevated level Similar increases were observed with another non-lysosomal, smooth membrane associated enzyme, leucine-2-naphthylamidase

The time dependence of the selective release of lysosomal enzymes caused by asbestos is shown in Fig 2 MP cultures exposed to asbestos (50 $\mu g\ ml^{-1}$) show no change compared to control cultures, in the distribution of a representative lysosomal enzyme, β-galactosidase, between cells and culture medium for the first 3 h By 4 5 h, however, a highly significant increase ($P < 0\ 01$) in the amount of β-galactosidase in the medium is seen Subsequently a rapid rise in the release of enzyme into the culture medium occurs so that by 24 h approximately 70% of the total enzyme activity is found in the culture medium As in the other experiments described in Table 1 and Fig 1, this release occurs with no detectable loss of cellular lactate dehydrogenase into the culture medium and significant increases in cellular levels of this enzyme at 24 h The delay in release is most probably due to the coating of ingested asbestos fibres with plasma proteins These proteins are removed within secondary lysosomes by proteolytic digestion thereby exposing the membrane reactive sites, probably magnesuim ions[19], on the asbestos fibres It is thought that the magnesium groups of asbestos interact with membrane sialoglycoproteins[20] and it is possible that such an interaction leads to the formation of protein clusters in membranes, a phenomenon observed during exocytosis in other systems[21] The toxic effect of silica on MP is well established and is attributed to the formation of hydrogen bonds between hydroxyl groups of silicic acid and membrane phospholipids Silica does not cause any selective release of lysosomal enzymes (P D , A C A , J A , A B and S W , unpublished), the appearance of acid hydrolases in the culture medium being accompanied always by lactate dehydrodgenase These observations support the concept that clustering of membrane proteins is an essential prerequisite to membrane fusion such as occurs during exocytosis[22]

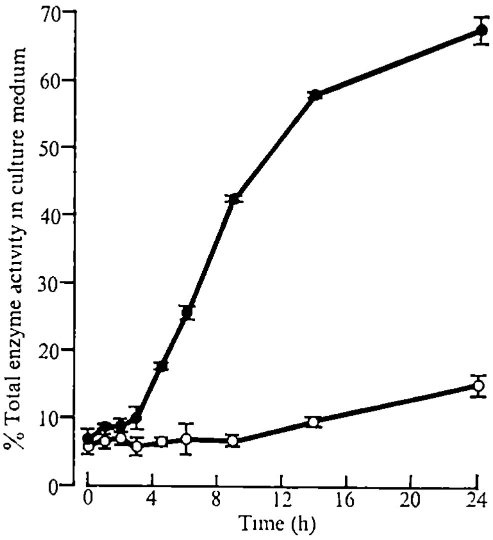

Fig 2 The time dependence of β- galactosidase release from MP induced by asbestos MP cultured in the presence (●) and absence (○) of asbestos (50 $\mu g\ ml^{-1}$) were removed at various times for enzyme assay Each value represents the mean ± standard deviation of four observations Experimental conditions as described in Table 1

The sensitivity of MP cultured *in vitro* to toxic particules such as asbestos and silica provides a simple and sensitive way of screening various particulate substances for possible toxic effects It is insufficient to monitor cell viability, however We have demonstrated that asbestos can release a large proportion of cellular content of MP acid hydrolases without detectable cell death It is essential to determine the distribution of lysosomal enzymes between cells and culture medium This type of estimation is particularly relevant since several agents which cause chronic inflammation *in vivo* induce selective release of lyososomal enzymes from MP *in vitro*[8–12], accordingly we have found (M Dym, P D , and A C A , unpublished) that asbestos produces an intense granulomatous reaction after intramuscular injection in mice

After intrapleural injection of asbestos, a marked mononuclear reaction is also observel, this subsides shortly before mesotheliomas appears[23], raising the possibility that the mononuclear phagocytes in the granuloma prevent the multiplication of malignant cells[24] so delaying the appearance of the tumour

The results reported here have relevance in at least four different fields of study First, the experimental system used provides a simple and sensitive *in vitro* test for the possible cytotoxicity of particulate substances Second, our observations on the selective release of lysosomal enzymes from MP by asbestos provide further support for the possibility that this phenomenon may be responsible for certain aspects of tissue damage seen in chronic inflammation[5,12] Third, the use of biologically active substances of defined chemical structure such as asbestos may provide a useful research tool for determining the nature of membrane constituents involved in changes which lead to membrane fusion[22] Fourth, some granuloma-inducing material other than asbestos might be administered by intrapleural injection to persons who have in the course of their occupations been exposed to asbestos so as to maintain a local granulomatous reaction which might prevent the appearance of mesotheliomas and bronchogenic cancer

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Modulation of corticotrophin-releasing factor release from hypothalamic synaptosomes

NERVE endings (synaptosomes) isolated from the mammalian hypothalamus contain the hypophysiotrophic hormones which regulate adenohypophysial function Edwardson *et al* have shown[1] that release of these neurosecretory substances can be elicited from incubated hypothalamic synaptosomes by electrical or potassium stimulation and the release mechanism has been shown[2] to be calcium dependent We report here that steroid hormones administered both *in vivo* and *in vitro* and neurotransmitter substances given *in vitro* modulate the release of corticotrophin-releasing factor (CRF) from synaptosomes prepared from rat and sheep hypothalamus

Adult rats were given corticosterone acetate in drinking water (20 μg ml^{-1}) for a period of 20 h (average dose, 300 μg per rat) before being killed Synaptosomes were prepared from the hypothalami by a modified[3] version of the method of Gray and Whittaker[4] and suspended in Krebs-bicarbonate medium containing 10 mM glucose Suspensions of synaptosomes were incubated and stimulated electrically in Warburg respirometer vessels fitted with concentric gold ring electrodes as described previously[1] After incubation the preparations were centrifuged and the supernatant medium was tested for CRF activity Paired halves of rat adenohypophyses were incubated in 1 ml of the medium and adrenocorticotrophic hormone (ACTH) release was measured by radioimmunoassay[5] The antiserum used was raised against the species common, steroidogenic α1-24ACTH and does not show significant cross reaction with pituitary melanocyte-stimulating hormone

Medium from stimulated synaptosomes prepared from untreated rats gave a 61% increase in ACTH release compared with medium from unstimulated preparations (Table 1) Pretreatment with corticosterone completely abolished this response This was due to absence of CRF rather than to an inhibitory effect of the medium on the pituitary response, since a test stimulus of 40 mU vasopressin added with the same synaptosome medium produced a 144% increase in ACTH release

Table 1 The effect of *in vivo* pretreatment with corticosterone on the release of CRF by synaptosomes from rat hypothalamus

Preparation	No of experiments	ACTH released (ng ml^{-1}) Unstimulated	ACTH released (ng ml^{-1}) Electrically stimulated	% Increase
Untreated	5	28 1 ± 4 3	45 9 ± 8 5*	61
Corticosterone pretreatment *in vivo*	6	20 0 ± 2 7	19 8 ± 2 4	0
Corticosterone pretreatment *in vivo* + vasopressin test *in vitro*	6	18 6 ± 2 0	45 5 ±.4 2	144

Values were determined by radioimmunoassay using α1-24 antibodies and represent ACTH released from paired halves of rat adenohypophyses during a 45 min incubation period at 37° C with synaptosome supernatant Corticosterone was administered in the drinking water for 20 h before preparation of synaptosomes (average dose 300 μg per rat) A dose of 40 mU ADH was added to medium from stimulated synaptosomes prepared from corticosterone pretreated rats to show that the response of the test pituitary was normal

*Wilcoxon test, $P < 0 05$

Table 2 The effect of steroids on the release of CRF produced by electrical stimulation of synaptosomes from the sheep hypothalamus

Hormone	Concentration (M)	No of experiments	ACTH released ng ml^{-1} Unstimulated	Stimulated	% Increase
None	—	10	28.5 ± 4.0	59.3 ± 7.5*	108
Corticosterone	7.5×10^{-7}	6	31.3 ± 4.2	36.3 ± 5.9	16
Cortisol	6.0×10^{-7}	5	49.2 ± 13.3	48.4 ± 13.1	0
Cortisol	6.0×10^{-8}	6	62.3 ± 8.2	62.9 ± 12.0	10
Cortisol	6.0×10^{-9}	5	49.1 ± 4.8	65.4 ± 8.1	33
Deoxcorticosterone	8.0×10^{-7}	6	34.7 ± 4.0	58.9 ± 8.7†	70
Deoxycortisol (Substance S)	8.5×10^{-7}	5	43.4 ± 7.5	88.2 ± 18.0‡	101
Progesterone	9.5×10^{-7}	3	42.1 ± 6.4	68.2 ± 13.1	62
Oestradiol	1.1×10^{-6}	3	37.6 ± 6.2	57.0 ± 8.5	52
Deoxycortisol + cortisol	8.0×10^{-7} 6.0×10^{-7}	3	21.3 ± 4.3	42.2 ± 6.0	99

Values were determined by radioimmunoassay, as explained in Table 1 (means ± s.e.m.). Steroids were present during a 35 min incubation of synaptosomes and electrical stimulation was for 25 min. Wilcoxon test, $^*P<0.005$, $^\dagger P<0.025$, $^\ddagger P<0.05$.

A direct action of corticosteroids on the nerve ending was shown in further experiments with synaptosomes from the hypothalami of sheep killed by exsanguination. This species was chosen because of the relatively large amount of hypothalamic tissue available compared with the rat, and the synaptosomes were prepared as described previously. Steroid hormones were added to synaptosome suspensions which were incubated for 15 min without stimulation, followed by 25 min electrical stimulation. Hormones were added to the controls after stimulation to eliminate any differences due to inhibition of ACTH release by steroid in the medium. Where solubility of the steroid hormones in medium did not permit direct addition to the synaptosome suspension, they were first dissolved in absolute ethanol, placed in the Warburg vessels and evaporated to dryness before the synaptosomes were added.

Medium from synaptosomes stimulated in the absence of steroids produced a 108% increase in ACTH secretion compared with medium from the unstimulated preparation (Table 2). This stimulation of ACTH release was reduced to 16% by the presence of corticosterone at 7.5×10^{-7} M and completely abolished by cortisol at the same concentration. These concentrations of steroid are comparable with amounts present in the circulation but may not be physiological because of the absence of the plasma proteins to which they are normally bound. Cortisol, the chief glucocorticoid in the sheep, did however, block CRF release at 6.0×10^{-8} M and even at 6.0×10^{-9} M produced a substantial inhibition. The small size of this effective dose, which approximates to the levels of unbound cortisol in blood measured during stress, and its action within a relatively short time suggest that the neurosecretory nerve terminal may be an important site of action in the fast feedback control of ACTH release by corticosteroids which has been described recently[6].

The results in Table 2 show that there is some variation in the control values for ACTH secretion. The assay procedure involved the use of paired halves of adenohypophyses for simultaneous testing of stimulated and unstimulated preparations, however, and a valid comparison can be made between these two groups using the Wilcoxon matched-pairs signed-ranks test.

Results obtained with other steroids, suggest that the inhibitory effect of CRF release may be of a relatively specific nature. Progesterone and 17β-oestradiol at a higher molar concentration than cortisol produced a partial inhibition of CRF release. It has been shown[7], however, that a hormone such as 17αOR-progesterone, which has no glucocorticoid activity, may suppress the stress-induced release of ACTH in rats. The failure of deoxycortisol, the immediate precursor of cortisol in adrenal steroidogenesis, to inhibit CRF release is of interest. Preliminary experiments have shown that after preincubation with deoxycortisol, cortisol is no longer effective in inhibiting the electrically

Table 3 The effect of neutrotransmitters on the release of CRF by synaptosomes isolated from the sheep hypothalamus

Test	Concentration (M)	No of experiments	ACTH release (ng ml^{-1}) Control	Experimental	% Increase
Electrical stimulation (E)	—	10	28.5 ± 4.0	59.3 ± 7.5*	108
Acetylcholine (E)	4.4×10^{-11}	5	45.3 ± 7.8	76.9 ± 13.3	69
Acetylcholine (E)	2.2×10^{-10}	5	44.7 ± 7.4	102.2 ± 22.0†	142
Acetylcholine (E)	4.4×10^{-9}	6	38.4 ± 6.1	123.7 ± 15.2*	223
Atropine (C, E) + acetylcholine (E)	2.9×10^{-9} 4.4×10^{-9}	6	34.7 ± 6.7	41.8 ± 7.3	21
Dopamine (E)	6.5×10^{-8}	4	25.9 ± 5.4	25.7 ± 3.5	0
Dopamine (C, E) + acetylcholine (E)	6.5×10^{-8} 2.2×10^{-10}	6	49.9 ± 3.8	62.6 ± 6.4	25
Dopamine (C, E) + electrical stimulation (E)	6.5×10^{-8}	6	48.3 ± 5.6	52.8 ± 2.3	9
Noradrenaline (C, E) + acetylcholine (E)	6.0×10^{-8} 2.2×10^{-10}	3	29.6 ± 1.7	69.6 ± 19.4	135
5-Hydroxytryptamine (C, E) + acetylcholine (E)	5.5×10^{-8} 2.2×10^{-10}	3	32.9 ± 5.0	79.7 ± 6.4	142

Values were determined by radioimmunoassay as explained in Table 1. Where a single neurotransmitter is indicated as the test, this was present in the experimental flask (E) only. Where the effects of neurotransmitters or atropine on the release of CRF evoked by electrical stimulation or acetylcholine were investigated the former agents were present in both control (C) and experimental (E) flasks. Neurotransmitters were present during the 35 min incubation of synaptosomes and electrical stimulation, when used, was for the last 25 min of this period. CRF release in the presence of noradrenaline and 5-hydroxytryptamine alone did not differ significantly from control unstimulated values. None of the transmitters at the concentration used had a direct effect on the pituitary response to medium from stimulated synaptosomes. Wilcoxon test, $^*P<0.005$, $^\dagger P<0.025$

stimulated release of CRF and the results suggest that competitive inhibition may occur for binding sites which exist at the level of the nerve terminal These results are in good agreement with those[5] obtained from *in vivo* studies on the interactions of corticosteroids involved in feedback control of ACTH secretion The findings are of particular interest in that they suggest a physiological action of a steroid hormone which does not involve a nuclear site

Further evidence that the neurosecretory terminal is an important target for substances involved in the modulation of the hypophysiotrophic activity of the hypothalamus was obtained when neurotransmitter substances were added to suspensions of synaptosomes prepared from the sheep hypothalamus (Table 3) Acetylcholine in concentrations varying from $4\ 4\times10^{-9}$ to 10^{-11} M stimulated the release of CRF in a dose-dependent manner The response was not enhanced by eserine (results not shown) but it was blocked in the presence of atropine ($2\ 9\times10^{-9}$ M) suggesting the existence of specific cholinergic receptors on the CRF containing nerve terminal Dopamine ($6\ 5\times10^{-8}$ M) blocked the release of CRF produced by acetylcholine or electrical stimulation Noradrenaline at similar concentrations caused only a partial inhibition of CRF release and 5-hydroxytryptamine was without effect The results are compatible with findings obtained in the whole animal[9-11] and with the isolated hypophysiotrophic hypothalamus[12] *in vitro* and are consistent with an excitatory cholinergic and inhibitory dopaminergic modulation of CRF secretion The effects obtained with acetylcholine and dopamine on synaptosomes probably require that axo-axonal endings be present in the median eminence if the results obtained *in vitro* are to to be considered as having any relation to what may be happening *in vivo* While it seems likely that such dopaminergic terminals exist[13] there is at present no anatomical evidence for a cholinergic synapse with neurosecretory neurones in the median eminence In view of the specificity and sensitivity of the effect obtained with acetylcholine, however, we suggest that humorally mediated neurotransmitter may be active at the nerve ending *in vivo* The findings obtained with steroids and neurotransmitters emphasise the potential importance of control mechanisms which may operate at the level of the neurosecretory nerve terminal in neuroendocrine integration

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Seasonal variation in sensitivity of guinea pig tissues to agonists

WE have observed a marked and consistent seasonal variation in the sensitivity of guinea pig tissue to acetylcholine (ACh) Ilea taken from guinea pigs during the summer months (May to September) were much less sensitive to the stimulant effect of ACh than those removed during the winter (November to February) Dawson, Hemsworth and Stockham[1] also noted that the response of the ileum to spasmogens varied widely at different times of the year, but they gave no specific details

The purpose of these preliminary investigations was to see whether two other smooth muscle stimulants, 5-hydroxytryptamine (5-HT) and potassium chloride (KCl), which act in different ways from that of ACh, showed a similar change in sensitivity The stimulant action of 5-HT is mediated mainly through stimulation of nervous elements causing the release of endogenous ACh, but it also has some direct action on the longitudinal muscle[2] KCl is believed to use a different source of calcium from that of ACh when causing contraction of smooth muscle[3]

To see whether heart muscle also showed different sensitivity in the summer and winter we studied the positive chronotrophic effect if isoprenaline on isolated guinea pig atria

Male guinea pigs weighing 250–500 g were used They were housed in uniform conditions, three per cage in rooms maintained between 22° C and 25° C throughout the year The animals were fed on a standard diet of food pellets, (Amber Ltd) mixture number 19350 The pellets were coated with absrbic acid, 1 mg g^{-1} The average daily intake of ascorbic acid was 50–60 mg per guinea pig

Guinea pigs were killed by cervical dislocation and a 4 cm piece of ileum was removed about 20 cm from the ileocaecal junction The ileum was suspended in a 5 ml isolated organ bath containing Krebs-Hensleit solution aerated with a mixture of 95% O_2 and 5% CO_2, at a tension of 1 5–2 g, and maintained at a temperature of 36°–37° C Responses of the ileum to the spasmogens were recorded isotonically with a Statham strain gauge on a multichannel polygraph

Isolated guinea pig atria were set up under the same conditions, but the temperature was reduced to 30°–31° C Dose-response curves were established to ACh, 5-HT and KCl on guinea pig ileum by adding the drug at 2 min intervals, the bath fluid was changed after each dose On isolated atria cumulative dose-response curves for the increase in chronotrophic effect were obtained for isoprenaline by adding increasing amounts of the amine to the bath and washing out only after a maximum increase in rate was achieved, as described by Weinstock *et al*[4]

The concentration of drug in each case required to give 50% of the maximum response (ED_{50}) was determined The winter and summer values, for the ED_{50} for ACh, 5-HT, KCl and isoprenaline are shown in Table 1

The change from summer to winter brought about a general increase in sensitivity (lower ED_{50}) of both cardiac and smooth muscle to adrenergic and cholinergic agents Moreover, the difference in responsiveness of the ileum in the winter from that in the summer was much greater for ACh and 5-HT than for KCl Such a change in response of smooth muscle to agonists is reminiscent of the nonspecific increases in sensitivity seen after de-

Table 1 Seasonal responses of isolated guinea pig tissues to various agonists

Tissues	Drug	Season	*n*	ED$_{50}$ mean ± s e (ng ml^{-1})	Level significant (between winter + summer values)
Ileum	Acetylcholine	Nov–Feb 1971–72	6	2 7 ± 0 8	0 001
Ileum	Acetylcholine	May–Sept 1972	13	40 0 ± 4 7	
Ileum	Acetylcholine	Nov–Feb 1972–73	9	6 5 ± 0 6	0 002
Ileum	Acetylcholine	May–Sept 1973	15	44 6 ± 10 2	
Ileum	Acetylcholine	Nov–Feb 1973–74	12	6 0 ± 1 1	
Ileum	5-Hydroxytryptamine	May–Sept 1973	6	1,250 ± 25	0 001
Ileum	5-Hydroxytryptamine	Nov–Feb 1973–74	8	16 9 ± 3 3	
Ileum	Potassium chloride	May–Sept 1973	6	0 79 ± 0 17 mg ml^{-1}	0 05
Ileum	Potassium chloride	Nov–Feb 1973–74	5	0 31 ± 0 06 mg ml^{-1}	
Atria	Isoprenaline	May–Sept 1973	8	3 60 ± 0 68	0 001
Atria	Isoprenaline	Nov–Feb 1973-74	5	0 184 ± 0 004	

centralisation or reserpine treatment, Westfall[5] found that the increase in response of the guinea pig vas deferens to noradrenaline and methylfurmethide (a cholinergic stimulant) was greater than that to KCl

Dawson, Hemsworth and Stockham[1] found that a daily intake of 50 mg of ascorbic acid in the diet of guinea pigs resulted in an increase in sensitivity of the ileum to spasmogens As in our study the animals received this amount of ascorbic acid daily throughout the year, however, we have to seek a different explanation for the seasonal supersensitivity

All our experiments were carried out in solutions of identical calcium and magnesium ion concentration, which suggests that the difference in seasonal response, like that after decentralisation, may be related to a change in some property of the membrane binding site for calcium It does not seem to be due to a change in blood level of thyroxine, since it has been shown that this hormone increases sensitivity of the guinea pig heart to catecholamines but decreases the responsiveness of the ileum to ACh (ref 6) It may, however, be related to cyclic enzymatic or other endocrinological changes Johansson and Santuria[7] reported that the guinea pig heart has a higher noradrenaline level in the summer than in the winter, which could explain the reduced sensitivity to exogenous isoprenaline They also found that serum calcium and potassium were higher in the winter than in the summer

We would be interested to hear whether a similar change in sensitivity of guinea pig tissues occurs in other laboratories, particularly in those in which winter falls in June-July and whether tissues from other laboratory animals are similarly affected

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Morphological evidence for an experimentally induced synaptic field

WHEN fibres in the central nervous system (CNS) of invertebrates and lower vertebrates are interrupted, regenerating axons grow beyond the lesion and form new synapses on denervated neurones In the leech CNS, however, we have seen synapses formed by the regenerating fibres at the site of the lesion The formation of synapses in regions where they are normally absent raises important questions about the manner in which axons grow to their targets and form orderly connections

The CNS of the leech consists of segmental ganglia and longitudinal connectives The ganglia have a cell rind formed by nerve cell perikarya and a central fibrous neuropile containing most of the synapses The neuropiles are joined together by the connectives, which include axons of interneurones and sensory nerve cells The axons (0 1–9 μm in diameter) run caudally and rostrally and form three longitudinal bundles of fibres associated with the cytoplasm of two giant glial cells The cells are found in the two lateral bundles and their nuclei lie between successive ganglia[1–4] Many of the nerve fibres are enclosed individually within invaginations of the giant glial cell processes, and synaptic junctions are rare in the nerve bundles

A lesion in a connective interrupts the neural pathways linking the ganglia[5,6] and damages the giant glial cells Transected axons soon grow across the lesion, however, and form a neuroma enclosing a different kind of glial cytoplasm Since the animals soon swim normally again, functional connections between the isolated parts of the nerve cord must be re-established during regeneration A plausible explanation for this would be that regenerating fibres grow along the injured connective, and synaptic connections are restored at the ganglionic neuropile We have found, however, that regenerating axons build up a new neuropile at the site of the lesion This neuropile includes a synaptic field very similar to that in the ganglionic neuropile

Twelve leeches of the species *Macrobdella decora* were anaesthetised with 8% ethanol and lesions were placed at the centre or at one end of the connective joining either ganglia 3 and 4 or 4 and 5 Each lesion was a discrete or extensive crush that often also involved the immediate walls of the haemocoelom which encloses the entire nerve cord Although lesions resulted in abnormal swimming, normal behaviour resumed within 4 weeks Animals survived in tanks with deionised water at 20–25° C and were killed 4, 19, 30, 55, 86 and 111 d after the operation We started examining 4-d-old neuromas because at this stage axons growing from both stumps of the severed connective met across the lesion

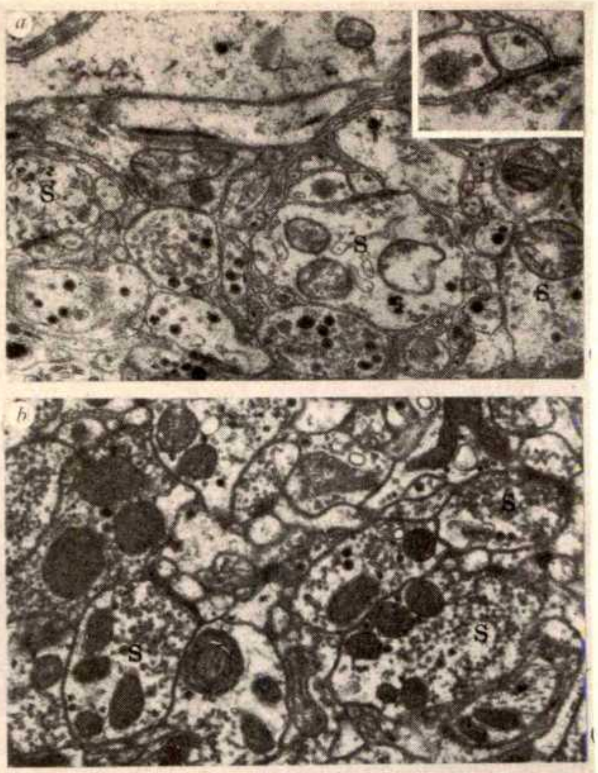

Fig. 1 Low magnification electron micrographs of 111-d-old neuroma (*a*) and normal ganglionic neuropile (*b*) that demonstrate the striking structural similarity between both kinds of neuropiles. Divergent types of synaptic clusters (S) are shown. Inset shows the specialised junction formed between the postsynaptic fibres. (*a*, × 16,280; inset, × 29,304; *b*, × 14,080).

Light microscopy showed that the lesions damaged part of the haemocoelom walls and underlying nerve connective, leaving cell debris which was ultimately removed by macrophages. Meanwhile many axons, growing from both ends of the lesion, advanced towards each other across the haemocoelom walls and formed a neuroma. Serial sections revealed the absence of giant glial cell cytoplasm across the nerve tissue, which consisted of many small and a few large fascicles near the twisted haemocoelomic canal.

Electron microscopy of neuromas of different ages confirmed the absence of giant glial cell cytoplasm, but revealed that of many small glial cells, not found in the normal connective. In very young neuromas (4 d), most immigrating glial cells appeared to be confined to the surface of regenerating tissue. In older neuromas, however, such cells were widespread.

The neuroma gradually transformed into a neuropile-type structure (Fig. 1*a*), with the nerve processes resembling those of the ganglionic neuropile (Fig. 1*b*). Thus, many neural profiles lacked a glial lining, or it extended only partly around their perimeter, leaving more direct surface contact between the nerve fibres than in the normal connective. Axons frequently formed axo-axonic synapses and contained many vesicular profiles of both granular (600–1,200 Å in diameter) and agranular (400 Å in diameter) types. The latter were particularly numerous in the presynaptic axons, mostly lying adjacent to the synaptic membrane. Here there was also electron dense material that was usually thicker than that associated with the postsynaptic membrane. The synaptic cleft measured approximately 180 Å in diameter (Fig. 2).

In both ganglionic and experimentally induced neuropiles, axo-axonic synapses were mostly in clusters, usually including one presynaptic and several postsynaptic fibres. Thus, the key feature of organisation of the synaptic clusters was divergence (Figs 1 and 2). When clusters included more than one afferent fibre, however, there was sometimes convergence. The extracellular space between postsynaptic fibres often narrowed and specialised neural junctions were formed (Fig. 1*a*).

Synaptic clusters were present in 4-d-old neuromas (Fig. 2*a*) and their numbers increased during the first weeks after the operation. The fact that a synaptic field was still present in the oldest neuromas we examined (111 d) is indicative of its nontransient character (Figs 1*a* and 2*b*).

What causes the regenerating nerve fibres of the leech to form an extraganglionic synaptic field at the site of injury? First, it seems likely that local conditions at the lesion favour direct interaction between growing axons. One such condition is the early absence of glial cell barriers throughout the regenerating tissue. This is associated with both the failure of the giant glial cells to repair damaged parts and the delayed invasion of the neuroma by glial cells. The competence of axons to synapse is probably associated with the early availability of new synaptic sites at the axon surface. These sites may arise, at least in part, as a consequence of collateral axon sprouting, which can be induced by, for example, partial denervation and nerve injury[7–12]. Some collateral axonal sprouts may come from undamaged nerve fibres. However, it is reasonable to suspect that most would originate from regenerating fibres growing across the lesion site. This view is supported by the fact that transected axons in the nerve connectives of the leech sprouted vigorously compared with those in the CNS of higher vertebrates[12,13]. Finally, there is evidence that axonal sprouts can form new synaptic junctions when they meet the receptive surface of a neurone or muscle cell[12,14].

What type of neurones form synapses at the neuroma? Many neurones of neighbouring ganglia are linked synaptically[6] and many of them recover their junctions after partial severance of a nerve connective[15,17]. Thus many neurones may re-establish at least some of their specific connections at the neuroma. This implies that many of the synapses would

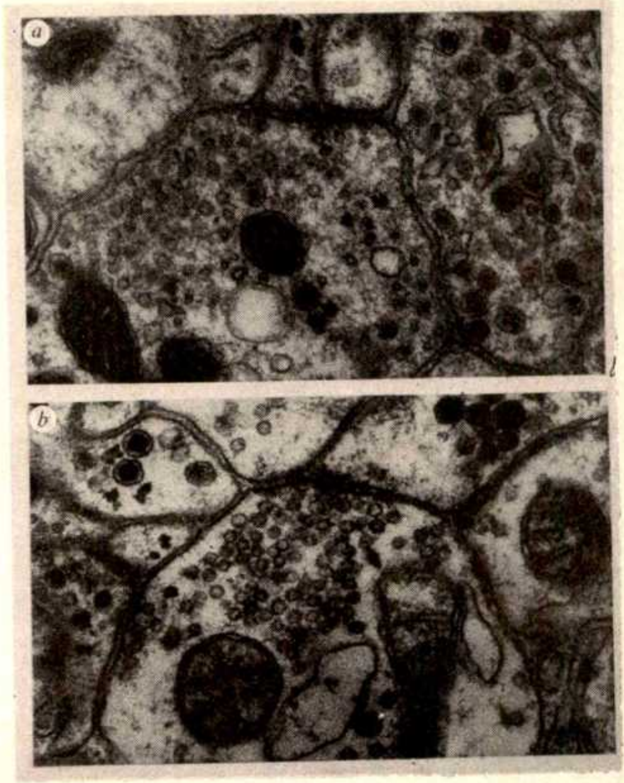

Fig. 2 High magnification electron micrographs of synaptic clusters in 4-d-old (*a*) and 111-d-old neuromas (*b*). In both cases, the cluster includes a single presynaptic fibre (× 40,480).

be formed between the central processes of neurones growing from opposite stumps of the severed connective. The fact that the conduction of action potentials is regained across the neuroma[15] and that some regenerating fibres grow beyond this structure (our unpublished work) suggests that axons also restore their specific connections at the ganglionic neuropile. Hence, a given pair of neurones of successive ganglia might renew their interconnections completely either at one or both neuropiles. Alternatively, many of the synapses found at the neuroma may be duplicates of those formed by the same regenerating fibres at the level of the ganglia. Thus, there would be a factor of redundancy in regenerated synapses. This might explain some consistent differences between regenerated and normal synapses with regard to the balance of excitation and inhibition[17].

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Effect of prostaglandins E_1, E_2 and $F_{2\alpha}$ on human blood coagulation

As prostaglandins are being used to induce labour and abortion in some women, it is necessary to know the effect of these compounds on the blood coagulation system. We have investigated this problem using plasma from normal women and prostaglandins E_1, E_2 and $F_{2\alpha}$ (Upjohn).

The prostaglandins, dissolved in absolute ethanol, were added to samples of the plasma to give a final concentration of 1, 10 and 100 ng ml^{-1}. Each 0.1 ml of plasma contained the equivalent of 1.0 ng of ethanol, which was added to similar samples of plasma during control runs of coagulation tests. After the addition of prostaglandins, aliquots of plasma were incubated for 1 h at 37° C to determine whether there was any conversion to prostaglandins A_1 or B_1[1,2]. While preparing 'platelet-rich plasma' we minimised the release of prostaglandin from the platelets. We used slightly modified versions of established methods to study blood coagulation in ten samples of plasma from each of ten subjects. To 0.2 ml of control and prostaglandin-treated plasma we added one of the following: thromboplastin, to measure prothrombin time[3]; cephaloplastin (Dade, Miami) to measure partial thromboplastin time[4], and thrombin (0.7 U per 0.1 ml, Fibrinex, Ortho) to measure thrombin clotting time. Percentage clot retraction was estimated by addition of 0.1 ml of cephaloplastin to 0.8 ml of platelet-rich plasma, followed by 0.1 ml of 0.1 M $CaCl_2$ in 1.0-ml graduated tubes into which were inserted applicator sticks. This mixture was incubated for 30 min at 37° C. After removal of the clot adhering to the stick, the remaining fluid was estimated as the percentage of the original 1.0 ml.

Plasma factors, V, VII, VIII, IX, X, XI and XII were determined by a modification of the test for prothrombin and partial thromboplastin time, using plasma from subjects deficient in the various factors as the vehicle for the clotting effect on control and prostaglandin-treated plasma. The plasma factor assays for the several prostaglandins *in vitro* were assessed at the 100 ng level only. Coagulation tests were also carried out on plasma from several pregnant women receiving prostaglandin $F_{2\alpha}$ or Pitocin for induction of labour, or Pitocin for abortion, and from women at the time of delivery (unpublished results of R. K. Laros).

None of the prostaglandins in concentrations of 1–100 ng ml^{-1} affected the coagulation tests or the plasma factor assays. Similarly, none of the plasma from the *in vivo* study (unpublished results of R. K. Laros) yielded any significant deviation from normal values for prothrombin time, partial thromboplastin time, thrombin clotting time or percentage clot retraction. *In vivo* studies on patients receiving prostaglandin $F_{2\alpha}$ as a means of inducing abortion also independently supports this conclusion[5]. None of the plasma samples converted the added prostaglandins to A_1 or B_1, although this has been described elsewhere[1]. Tables 1 and 2 summarise the *in vitro* findings.

Table 1 Effect of prostaglandins E_1, E_2 and $F_{2\alpha}$ on coagulation tests on human blood plasma

Prostaglandin	ng	Mean* PT (S)	Mean† PTT (S)	Mean‡ TCT (S)	Mean§ clot retraction
E_1	1	10.2	31.3	8.8	83.0
E_1	10	10.1	30.3	8.8	80.0
E_1	100	10.2	32.5	8.8	70.0
E_2	1	10.1	32.0	8.3	80.0
E_2	10	10.3	32.2	8.8	80.0
E_2	100	10.3	32.5	8.8	85.0
$F_{2\alpha}$	1	10.0	31.0	8.8	85.0
$F_{2\alpha}$	10	10.2	32.0	8.8	75.0
$F_{2\alpha}$	100	10.3	31.0	8.3	80.0
Mean Control		10.2	30.5	8.8	80.0

Clot retraction is expressed as percentage of control. PT, Thromboplastin time; PTT, partial thromboplastin time; TCT, thrombin clotting time.
* Range, 10–16 s.
† Range, 30–33.0 s.
‡ Range, 8.0–9.0 s.
§ Range, 70.0–90.0%.

Prostaglandins E_1 and E_2, apparently at physiological (1.0 ng ml^{-1} plasma) as well as the pharmacological levels we used, do not affect the clotting mechanism other than by inhibiting platelet aggregation and adhesiveness on the part of prostaglandin E_1. Blood samples from patients obtained before infusion of prostaglandin $F_{2\alpha}$ and at delivery, when prostaglandin concentrations are maximum and immediately *post partum* were relatively unchanged, although significant coagulation abnormalities developed in patients being aborted with the aid of Pitocin (unpublished results of R. K. Laros).

Table 2 Effect of prostaglandins E_1, E_2 and $F_{2\alpha}$ on plasma coagulation factors tests

Prostaglandin	ng	Mean factor V	Mean factor VII	Mean factor VIII	Mean factor IX	Mean factor X	Mean factor XI	Mean factor XII
E_1	100	104	100	73 0	90 0	100	102 0	88 0
E_2	100	101	147	117 0	87 0	113	83 0	92 0
$F_{2\alpha}$	100	104	100	77 0	90 0	100	96 0	106 0
Control (1 ng ethanol)		100	100	100 0	100 0	100	100 0	100 0

Values are expressed as mean percentage of control
Ranges for factors V, 100–108, VII, 100–150, VIII, 70-110, IX, 90–100, X, 100–106, XI, 100–104, XII, 80–100

Prostaglandins seem to affect haemostasis through the platelet The ability of prostaglandins to interfere with platelet aggregation has been well established and may represent one of the major effects of prostaglandins on the clotting mechanism, although less well documented Ferri *et al*[7] noted a shortening of the recalcified clotting time in citrated rat blood, suggesting the development of a hypercoagulable state Kloeze[9], on the other hand, also working with rat plasma could not alter the prothrombin time, but changed the thromboelastogram pattern of prostaglandin-containing blood, an effect probably related to platelet function In contrast to our findings, Murer[9], comparing different systems of clot retraction, noted an inhibitory effect of prostaglandin E_1 on diluted calcium-deficient plasma solutions clotted with thrombin Addition of calcium reversed the inhibition, resulting in normal clot retraction Suspensions of washed platelets where clots were formed by fibrin monomers and calcium also were inhibited by prostaglandin E_1 and were not improved by further addition of calcium[9] Addition of calcium to the platelet-rich plasma alone in our system was unaffected by prostaglandins E_1, E_2 and $F_{2\alpha}$

Haemostasis therefore does not seem to be altered significantly by prostaglandins E_1, E_2 and $F_{2\alpha}$, and thus haemorrhagic or thrombotic complications would not be expected to represent a hazard in their therapeutic use

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Biochemical marker in dominantly inherited ectodermal malformation

ALTHOUGH several hundred dominantly inherited human malformations have been described[1], the mode of action of the mutant genes responsible for them is, in nearly all cases completely unknown, because of the lack of suitable biochemical markers Here we describe a well defined biochemical abnormality in the keratin of mice bearing the dominantly inherited Naked trait

The mouse phenotype known as Naked (N) has been described elsewhere[2–4] In the heterozygote, the hair breaks off close to the root causing patchy depilation which begins around the eyes and spreads to the tail Before depilation is complete, hair growth begins again on the head, and the mouse grows successive waves of hair passing from head to tail, reflecting the normal moult pattern Mice homozygous for this mutation, have almost no hair or nails, and usually die within 10 d of birth

We have investigated the amino acid composition of Nn hair and found it to be significantly different from that of wild type hair We used mice of the NS-FR strain, inbred by brother–sister mating for 57 generations with forced heterozygosis for the N and albino genes Eight mice were examined Four were heterozygous Naked (Nn) and four were homozygous for the normal allele (nn) Each of the groups comprised two black and two albino mice

Three replicate samples of hair shaft (2 mg each) were taken from each mouse, washed with water and defatted in petroleum ether, dried and added to 5 ml of redistilled 6 N HCl in a hydrolysis tube, which was then evacuated, sealed and heated at 110° C for 24 h The contents of the tubes were then evaporated under vacuum to dryness and the residues were dissolved in *p*H 2 2 citrate buffer at a concentration of 0 15 mg of protein ml^{-1} The amino acid composition of the resulting solution was determined on a Jeol analyser A standard mixture of amino acid was analysed at every sixth run

The amino acid composition of the hair samples (Table 1) is expressed as the percentage of moles represented by each amino acid An analysis of variance was carried out to determine the effect of the N mutation, the albino mutation and the interaction between them The albino gene causes no significant change nor does it interact significantly with the N mutation The latter, however, causes two significant changes in amino acid composition a 30 4% reduction in the tyrosine content (3 23 against 4 67 mol %) and a 20 4% reduction in the glycine content (8 41 against 10 51 mol %) Since the probability of finding *n* or more differences, at significance *P* or less among *N* variables is $p^{n} N!/(N-n)!n!$, the probability that this is a chance finding is 0 00015 A few samples of hair were hydrolysed for 48 and 72 h to estimate rates of amino acid destruction The corrected values for glycine (mol %) are normal 9 37, naked 7 72, and for tyrosine normal 4 58, naked 3 25 The differences between genotypes thus persist when values are corrected for destruction For these reasons, observed reductions in tyrosine and glycine are almost certainly the result of the mutation

In 1964, Dedeurwaerder *et al*[5] extracted from wool a protein fraction containing 27 mol % of glycine and 11 mol % of tyrosine The proteins comprising this fraction are now known as the high-gly, high-tyr (HG) proteins It was apparent from electron microscopic studies of the wool after extraction of the HG proteins, that they are located in the intercellular membrane complex In fact, the removal of HG proteins releases intact cells from the fibre A complete sequence of an HG protein has now been published[6] It has a molecular weight of 6,950 and contains 23% glycine and 19% tyrosine

It has been shown that the HG protein content of a hair is roughly proportional to its tyrosine content[7] and, extrapolating from keratin samples whose tyrosine content and HG protein content have both been determined, we estimate that murine hair contains 20–25% by weight of HG proteins Murine hair contains more tyrosine than any animal hair so far investigated[8]

The composition of murine HG protein has been determined[8] It contains roughly three times as much glycine and four times as much tyrosine as whole hair A defi-

Table 1 Amino acid composition of mouse hair

Amino acid (mol %)	Control				'Naked'				F values		
	Black		Albino		Black		Albino		Interaction	Albino	Naked
	Animal										
	1	2	3	4	5	6	7	8			
Lysine	3 00	2 97	3 43	3 30	3 60	4 13	3 63	3 96	2 47	1 81	9 17§
Histidine	1 03	1 03	1 00	0 97	1 00	1 10	1 03	1 03	0 40	1 60	1 60
Ammonia	11 20	10 30	9 87	10 67	11 90	13 03	9 50	11 00	1 75	4 25	1 69
Arginine	5 10	5 07	5 03	5 47	4 80	4 77	5 00	5 03	0 09	3 35	5 95
Aspartic acid	4 90	4 87	5 13	4 83	4 87	4 97	5 00	4 87	0 23	0 46	0 01
Threonine	4 07	4 10	4 10	3 97	4 20	4 13	4 40	4 13	0 95	0 11	4 25
Serine	7 47	7 63	7 10	7 20	7 87	7 17	8 10	7 40	1 58	0 11	1 26
Glutamic acid	11 33	11 53	11 90	11 13	12 20	12 30	12 03	11 70	1 16	0 48	7 27
Proline	5 83	5 86	6 00	5 73	6 10	6 13	6 67	6 50	8 01§	9 24§	38 25†
Glycine	10 33	10 47	10 53	10 73	8 13	8 50	8 50	8 50	0 05	3 61	370 0*
Alanine	4 00	4 07	4 13	4 07	3 90	3 93	3 93	3 97	0 40	3 60	25 60‡
Half cystine	11 53	11 90	11 13	11 46	12 70	11 03	12 93	12 76	2 58	0 41	3 81
Valine	4 03	4 00	4 20	3 97	4 07	4 27	4 20	4 30	0 01	0 85	3 80
Methionine	0 90	1 10	0 97	0 97	1 33	1 07	0 93	1 00	1 38	2 46	1 38
Isoleucine	2 40	2 30	2 43	2 27	2 50	2 50	2 50	2 47	0 03	0 03	8 26§
Leucine	5 87	5 70	5 97	5 70	5 53	5 70	5 77	5 60	0 01	0 35	2 60
Tyrosine	4 37	4 63	4 67	5 00	3 00	3 10	3 43	3 40	0 02	10 14§	170 0*
Phenylalanine	2 53	2 37	2 47	2 47	2 20	2 30	2 37	2 33	0 71	1 40	103 1§

Studies were performed on two mice in each genotype category The values represent the means of three replicate analyses *F* values are with 1 and 4 degrees of freedom in numerator and denominator respectively

* $P <0\ 001$

† $0\ 001 < P <0\ 005$

‡ $0\ 005 < P <0\ 01$

§ $0\ 025 < P <0\ 05$

ciency of HG protein would therefore explain the low tyrosine and glycine contents of naked hair A consideration of other amino acids is also consistent with an HG deficiency There is twice as much phenylalanine in HG protein as in whole hair and there is a deficiency of this amino acid significant at the $P=0\ 05$ level, in naked hair On the other hand there is less leucine, lysine and proline in HG than in whole hair and the relative amounts of these amino acids are increased in naked hair ($P<0\ 05$, $<0\ 05$ and $<0\ 005$, respectively) The amino acids which are present in HG protein and in whole hair, in roughly equal amounts are unchanged in naked hair, for example, histidine We can make a rough estimate of the amount of HG protein which would have to be removed from normal hair If normal hair, mutant hair and HG protein contain respectively n, m and h mol % of a given amino acid, then

$$(n-m)/h-m)=p,$$

where p is the proportion of HG protein lost[9] The mean value of p obtained by considering several amino acids is 0 12 Thus, an amount of HG protein equal to about 12% of the total hair by weight is missing in the naked mutant

Since the HG fraction is very heterogeneous[8], this almost certainly represents the loss of several molecular species

The original reason for investigating this mutation in the mouse was its close resemblance to the dominantly inherited human disease, hydrotic ectodermal dysplasia[10] The amino acid changes in this disease are quite different, however Ectodermal dysplasic hair contains less cystine, proline and serine and more tyrosine and phenylalanine than normal hair and its glycine content is unchanged It is probably caused by the loss of a matrix component[11-13] The entirely different nature of the two superficially similar phenotypes underlines the danger of accepting animal diseases as models for human ones without a knowledge of the underlying mechanisms

The results also demonstrate the advantage of using isogenic, animal strains The amino acid composition of human hair, both normal and dysplasic is very variable and the difference between them is much less clear, presumably as the result of the action of modifying genes The genotypes of the mice, on the other hand, differ only at the locus whose effects we wish to observe

Meanwhile, an exploration of the protein fractions in the Naked mouse hair and comparison with those in normal hair should further elucidate the gene action in this mutation

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DNA polymerase activity determined by the ultraviolet-protecting plasmid, R-Utrecht

CERTAIN plasmids, including the colicin factor *Collb* and the drug resistance transfer factor R-Utrecht, reduce the susceptibility of wild-type cells of *Salmonella typhimurium* to the lethal effects of ultraviolet irradiation while at the same time increasing their susceptibility to the mutagenic effects[1,2] These alterations in ultraviolet response may result from plasmid-borne genes whose products participate in repair of single-strand gaps in DNA (ref 2) Here I present evidence which indicates that at least part of the ultraviolet protection conferred on wild-type cells by R-Utrecht can be attributed to a plasmid-coded DNA polymerase which participates in repair of single strand gaps

The strains used are derivatives of *S typhimurium* strain M7471, which is of the 'FIRN' wild-type and differs from LT2 by absence of fimbriae and failure to ferment inositol and rhamnose[3] The parent strain, SL4525, has the following genotype M7471 (*ColEl-30*) *leu-1051* *malB479* *cysI1173* *hisC527* (amber) *gal-459* Strain SL4702 is a derivative of SL4525 detected as a clone which failed to release colicin El This strain shows increased sensitivity to ultraviolet and ionising radiation because of a deficiency in the enzyme DNA polymerase I, and the mutation it carries has been designated *polA1* (ref 4) Strains SL4525/R-Utrecht and SL4702/R-Utrecht were obtained by selection of clones resistant to tetracycline following conjugation with *S typhimurium* strain SL1156/R-Utrecht (an LT2 derivative) The characteristics of this strain and of the R-Utrecht plasmid have been described previously[2]

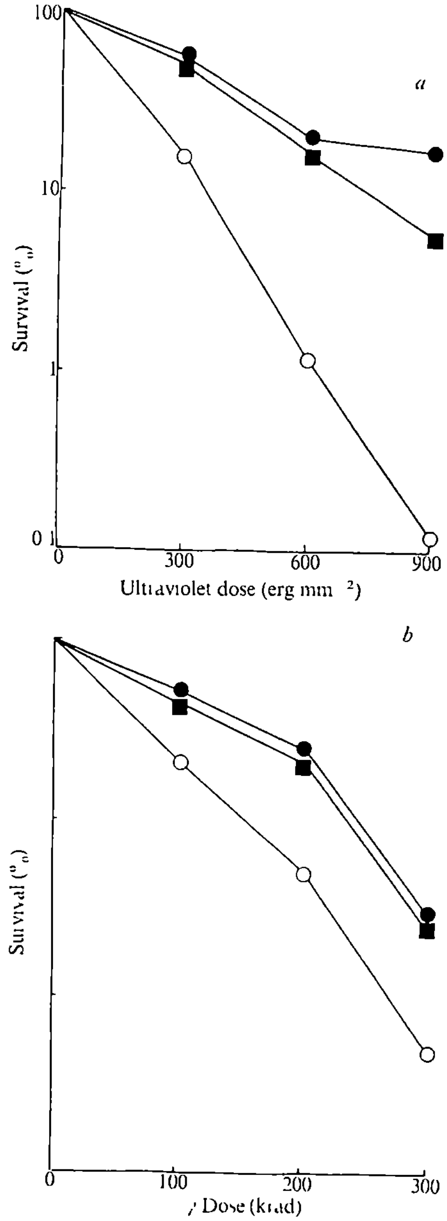

Fig 1 Host–cell reactivation of preparations of bacteriophage ES18 exposed to, *a*, ultraviolet irradiation and, *b*, γ-irradiation Phage suspended in saline were irradiated, diluted in broth and titrated on the various strains by the drop-on-lawn method[4] ■, SL4525 (POL$^+$), ○, SL4702 (*polA1*), ●, SL4702/R-Utrecht (*polA1*/R-Utrecht)

Among other properties, the *polA1* strain of *S typhimurium* (SL4702) shows reduced ability to effect host–cell reactivation (HCR) of bacteriophages which have been damaged by ultraviolet or ionising radiation (D G M, unpublished) Figure 1 confirms this finding, showing that preparations of the *Salmonella* phage ES18 which have been exposed to ultraviolet or γ-irradiation plate with lower efficiency on strain SL4702 than on the wild-type parent strain SL4525 Tests with derivatives of these strains carrying the R-Utrecht factor showed that this plasmid has no detectable effect on the HCR capacity of strain SL4525 for either phage preparation (results not shown), and that this plasmid increases the HCR capacity of strain SL4702 for both ultraviolet and γ-irradiated phages to a level approximately equal to that of the wild-type parent strain SL4525 (Figs 1*a* and *b*)

Those results suggest that R-Utrecht provides cells of the *polA1* strain with a plasmid gene product which can participate in repair of single-strand gaps produced in phage DNA either indirectly as a result of excision of ultraviolet-induced photoproducts or directly as a result of exposure to ionising radiation, and that this gene product is therefore functionally equivalent to the chromosomal *polA*$^+$ gene product (DNA polymerase I) On this hypothesis, cells of a *polA1*/R-Utrecht strain of *S typhimurium* would be expected to contain an enzyme capable of carrying out the process known as repair resynthesis or repair replication (that is, resynthesising DNA in the region of a DNA strand gap using the intact opposite strand as a template) The chromosomally-determined enzyme believed to be mainly responsible for this process in normal cells is of course DNA polymerase I, an enzyme whose activity can be detected even in crude assay systems and certainly much more readily than either of the other two known cellular DNA polymerases (II and III)[5-7]

Assays for DNA polymerising activity were therefore performed on crude extracts obtained by treatment of strains SL4702 (the *polA1* mutant), SL4702/R-Utrecht, and SL4525 (the *polA*$^+$ parent strain) As expected from previous work[4] the results (Fig 2) show first that crude extracts of strain SL4702 contain no detectable DNA polymerising activity They also show that the derivative of strain SL4702 carrying R-Utrecht has about 25% of the DNA polymerising activity found in extracts of the wild-type parent strain SL4525 It therefore seems clear that a new DNA polymerising activity is associated with the acquisition of R-Utrecht by strain SL4702 Further experiments have shown that the continued presence of R-Utrecht is necessary for this activity to be found in cells derived from strain SL4702, since spontaneous R$^-$ segregants of the R$^+$ strain have no detectable polymerase activity

It has been assumed so far that the enzyme which contributes to DNA repair in *polA1* cells carrying R-Utrecht and which is responsible for the DNA polymerising activity found in such cells is directly coded by the plasmid There are other possibilities, however For example, the effect of the plasmid could be to derepress a chromosomal gene specifying a DNA repair enzyme which has DNA polymerase activity If so, the chromosomal gene most likely to be implicated is the *polA* gene itself, since detection of either of the other two known cellular DNA polymerases (II and III) in the crude assay system used here is improbable It then becomes necessary to assume that the *polA1* mutation in strain SL4702 leaves the *polA* gene product with some residual DNA repair and polymerising activity which can be amplified by plasmid-directed derepression In fact, it is necessary to extend this assumption

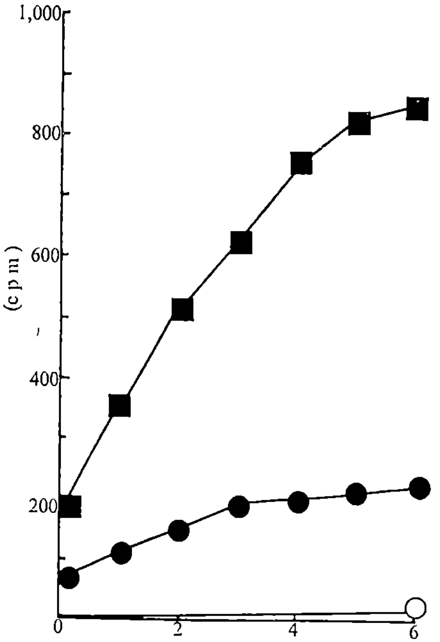

Fig. 2 Assays for DNA polymerase activity of strain, ■, SL4525 (POL$^+$), ○, SL4702 (*polA1*), and, ●, SL4702/R-Utrecht Bacteria grown on the surface of a solid medium were suspended in 0 1 M Tris, 0 01 M $MgSO_4$ (*p*H 7 4) at a concentration of about 1×10^{10} ml^{-1} Crude extracts of these suspensions were then prepared by sonication in a 150 W MSE ultrasonic disintegrator The resulting extracts were centrifuged at 1,000*g* for 10 min To 0 9 ml of each supernatant, 0 1 ml sonicated calf thymus DNA was added (final concentration 20 μg ml^{-1}), and, 5 min later 0 3 ml triphosphate solution (final concentrations 100 nmol ml^{-1} dGTP, dCTP, dATP and 0 6 nmol ml^{-1} ^{3}H-TTP) Samples of 0 02 ml were taken from each reaction mixture and dried on Whatman No 1 filter paper disks (2 5 cm diameter) The disks were immediately placed in cold 5% trichloroacetic acid, 1% sodium pyrophosphate for at least 2 h then rinsed with 95% ethanol, dried, and counted in a scintillation counter

to four other independently-isolated *polA* mutants of *S typhimurium* since R-Utrecht has similar effects in all five strains It seems equally improbable that a plasmid-determined suppressor of any known type could explain the effects of R-Utrecht in the five different *polA* mutants, especially since one of the mutants (*polA3*) is known to be suppressible by ochre and amber suppressors whereas the others are known not to be (D G M, and M R Beazer, unpublished)

On balance, therefore, it seems highly likely that the R-Utrecht plasmid codes directly for a DNA polymerase which resembles the chromosomal DNA polymerase I in being readily detectable in assays performed on crude cell extracts and in contributing to repair of single-strand gaps in DNA Such an enzyme presumably provides R$^+$ derivatives of wild-type strains of *S typhimurium* with additional DNA polymerising activity, and thus may account, at least in part, for their increased resistance to the lethal effects of ultraviolet and ionising radiation This would seem to be a reasonable assumption, since there is evidence from other studies that strains of *S typhimurium* which have increased levels of DNA polymerase I activity (probably as a result of mutation of chromosomal genes in this case) show increased resistance to both ultraviolet and ionising radiation[8]

Plasmid-coded DNA polymerases may well have other functions quite apart from their role in DNA repair For example, they may be responsible for the special type of DNA replication (known as transfer replication) which is thought to be involved in the transfer of plasmid (and in some cases chromosomal) DNA from a donor to a recipient cell during conjugation[9], or alternatively they may be involved in some aspect of the replication and stable maintenance of plasmid DNA within the bacterial cell In the latter situation, possible roles which might be attributed to plasmid-coded DNA polymerases will depend upon whether they resemble the multifunctional DNA polymerase I of *Escherichia coli* in properties other than just ease of detection in crude assay systems (for example, in having exonuclease activities as well as their polymerase activity) Thus, until a particular plasmid enzyme has been studied in detail, the exact role (or roles) which it plays in the cell must remain a matter for speculation

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Altered rate of DNA replication in ageing human fibroblast cultures

SINCE the demonstration that diploid human fibroblasts have a limited lifespan in culture[1,2] and that the lifespan in culture is a function of the age of the donor[2,3], many explanations of fibroblast ageing have been offered[4] The production of error-containing enzymes by either an 'error catastrophe'[5,6] or somatic mutation has been suggested as one possible cause[4,7] Errors in the enzymes which replicate the cellular DNA might have the gross effect of slowing the rate of DNA replication Here we examine the rate of DNA chain elongation and the distance between DNA synthesis initiation sites in senescent and control fibroblasts by DNA fibre autoradiography

Asynchronous cultures established from the male foetal lung fibroblast strain MRC-5[7,8] were pulse-labelled with ^{3}H-thymidine and prepared for DNA fibre autoradiography[9–11] Parallel experiments were performed with a visibly senescent culture (culture passage 58) and a control nonsenescent culture (passage 16) The strain MRC-5 generally becomes visibly senescent after about 50 passages and ceases growth after 60–70 passages[7]

Tandem arrays of grain tracks, similar to those observed in other eukaryotic systems[9–13], were observed in autoradiographs prepared from both passage 58 and passage 16 cells The length of individual tracks in these arrays from cells labelled for 10, 20 and 30 min is shown in Fig 1 For each of the three pulse experiments, the length of tracks from the passage 58 cells were significantly smaller ($P<0\ 05$) than those from passage 16 cells The average track lengths for passage 58 and passage 16 cells were respectively for the 10 min pulse, 7 μm and 10 μm,

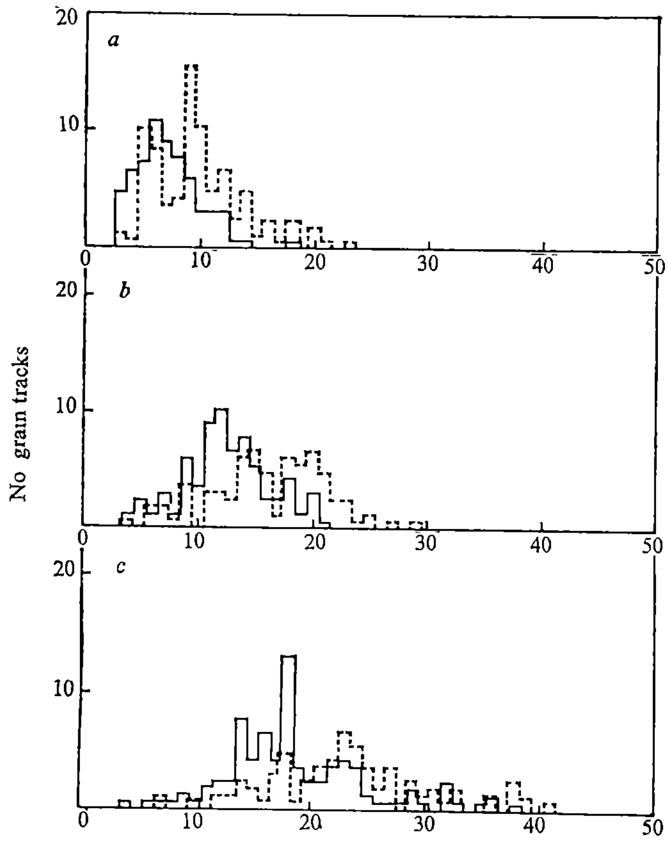

Fig 1 Histograms of lengths of labelled segments of DNA from senescent and non senescent cultures. Methods for labelling the DNA and fibre autoradiography followed procedures of Lark *et al*[11] and Huberman and Tsai[16] with minor modifications. Cultures of MRC-5 (passage 58 and passage 16 cells previously tested for mycoplasma contamination) were seeded before labelling into 50 mm plastic Petri dishes containing 10 ml of Eagle's basal medium (BME), supplemented with 10% foetal calf serum, nonessential amino acids, and penicillin (100 units ml^{-1}), streptomycin (100 μg ml^{-1}). Approximately 10^5 cells were labelled in 2 ml of the above medium containing 200 μCi of ^{3}H-thymidine (56 Ci $mmol^{-1}$, Amersham) for various pulse times (10, 20, 30 min, 1 h or 3 h). All pulses were done at 37° C. The pulses were terminated by washing with cold isotonic saline containing fluorodeoxyuridine (0.1 μg ml^{-1}) and the cells removed by trypsin (0.125% with 0.1 μg ml^{-1} fluorodeoxyuridine) treatment at 37° C for 15 min (ref 16). The DNA from approximately 5,000 cells was spread on glass slides[11,16]. The slides were rinsed with 5% trichloroacetic acid and ethanol[11,16] and covered with Kodak AR-10 stripping film[10]. Exposure time was 3–4 months. Track lengths were measured with an ocular micrometer. Slides were coded before measurements to exclude the possibility of bias in scoring. ——, Passage 58, -----, passage 16. *a*, 10 min pulse, *b*, 20 min pulse, *c*, 30 min pulse.

for the 20 min pulse, 12 μm and 16 μm, and for the 30 min pulse, 19 μm and 23 μm. The tracks labelled in 1 h and 3 h labelling periods were very long, often exceeding 100 μm in length, in autoradiographs from both passage 58 and passage 16 cultures. These were not further characterised.

The length of grain tracks produced during a pulse time which is short relative to the time necessary to complete replication of a replicating unit is primarily a function of the rate of chain elongation of DNA (ref 10). The results, therefore, suggest that DNA chain elongation is slower in senescent cells. It is unlikely that the difference in track lengths represents differential stretching of DNA during preparation of autradiography since the grain density of autoradiographs from passage 58 and passage 16 cells was not significantly different. It is also unlikely that the difference in track lengths represents a difference in the time required for equilibration of precursor pools. No measurable gradients of grain density were observed in grain tracks from either passage 58 or passage 16 cells. The difference in track lengths probably does not reflect an age-independent difference in the rate of DNA synthesis of different sublines of cells. Independent experiments with different sublines of senescent (passage 60) and nonsenescent (passage 20) cells showed the same difference in the rate of DNA chain elongation (T.D.P., unpublished). It should be pointed out that a 25% reduction in the rate of DNA chain elongation in senescent cells does not necessarily imply that the period of DNA synthesis (the S period) is 25% longer in senescent cells. Preliminary measurements of the length of the S period (R.A.F., G.M.T., T.D.P., unpublished) have indicated no difference between senescent and nonsenescent cells.

The rate of chain elongation as estimated by the length of grain tracks produced in a pulse-labelling experiment is only an approximation of the true rate of synthesis[10,12]. Initiation of DNA synthesis, termination of DNA synthesis and fusion of neighbouring replication units during the pulse are possible sources of error. Nevertheless, the calculation of the replication rate from the 10 min pulse experiment suggests that the rate of DNA chain elongation in nonsenescent human diploid fibroblasts, 0.5 μm min^{-1}, is similar to the rate of DNA synthesis in other mammalian systems[9,10,13]. For this calculation, it was assumed that DNA synthesis in human diploid fibroblasts is bidirectional from each initiation site as in other eukaryotic systems[10,12].

The distance between initiation sites along the chromosome was measured by determining the centre-to-centre distance between adjacent tracks within a tandem array of tracks[10]. There was no significant difference in the spacing of these sites in passage 58 and passage 16 cells (Fig 2). Most of the centre-to-centre distances were between 15 μm and 60 μm. Similar distances have been found in Chinese hamster ovary cells[10].

One possible reason for the slower rate of DNA elongation is that a proportion of the enzyme molecules in enzymes required for DNA replication is defective in senescent cells. Holliday and Tarrant[7] reported that senescent cultures of MRC-5 contained as much as 25% heat labile glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase and suggested that the heat lability was the result of misincorporation of amino acids during protein synthesis. If such misincorporation occurs in DNA polymerase, then even a small proportion of altered molecules could have a significant effect on the rate of chain elongation. One molecule of DNA polymerase at the replication fork may synthesise a small stretch of DNA (ref 14) (for example, one Okazaki fragment). An occasional defective

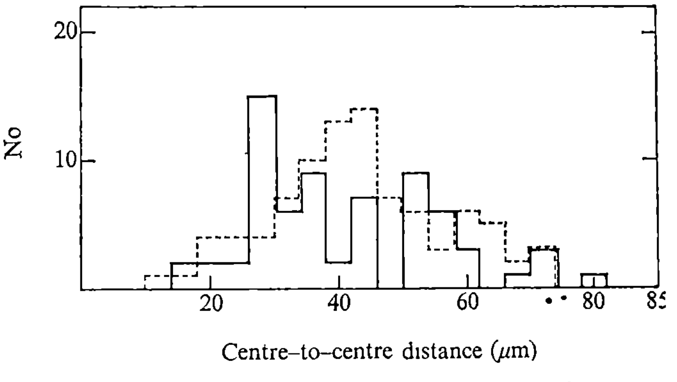

Fig 2 Histogram of the centre-to-centre distances between adjacent tracks in a tandem array. The autoradiographs were from the experiment described in the legend to Fig 1. The centre to-centre distances did not differ in the 20 min and 30 min pulses and the data from these experiments were pooled. ——, Passage 58, -----, passage 16.

polymerase molecule which binds normally may block replication for a significant length of time before being replaced by a normal molecule Faulty DNA polymerase molecules would also be expected to have 'mutator' activity[7], errors in replication would accumulate irreversibly and may be a major cause of cell death

The slower rate of chain elongation in senecent cells is not necessarily the result of defective DNA polymerase molecules It might also reflect smaller pools of DNA precursors or energy limitations Slow DNA replication as a consequence of limited precursor pools could be detrimental to the cell, since it has been observed that thymine-requiring strains of *B subtilis* grown in limiting concentrations of thymine have a mutation rate several orders of magnitude higher than the background rate[15] Slow DNA synthesis may also be a more general factor in cell death For example, completion of synthesis of certain replicons may be required to trigger other essential events in the cell cycle Slowing the rate of DNA synthesis could disrupt these control mechanisms

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Control of translation of globin mRNA in embryonic cells

WHEN mouse or rabbit globin mRNA is injected into oocytes of *Xenopus laevis* both α and β mRNAs are translated but only about one fifth as much α globin is synthesised as β globin[1–3] On the other hand, the same message sample results in about equal amounts of α and β globin synthesis in a reticulocyte cell-free system and is believed to contain roughly equal amounts of α and β globin mRNA Giglioni *et al*[3] have shown that if haemin is injected into oocytes at the same time as globin mRNA, there is an equal ratio of α β globin synthesis, and their experiments strongly suggest that this is achieved by increasing the efficiency of translation of α globin mRNA These experiments thus identified a factor which has a different effect on two kinds of mRNA, and further demonstrated that the microinjection of living cells can reveal regulatory effects not readily seen in the cell-free systems used so far The experiments reported here were also carried out by injecting mRNA into frog cells They show that during development a component is formed that alters the ratio of α β rabbit globin synthesis, a change not seen when mouse globin mRNA is injected This is therefore a second example of a translational factor revealed by a living cell assay system This component shows an even higher degree of selectivity in its action than haemin

Rabbit or mouse globin mRNA is translated into globin when injected into fertilised frog eggs[4–6], which differ in several respects from oocytes Mouse mRNA yields a low, although somewhat variable, ratio of α to β globin synthesis in eggs and embryos of all stages (Table 1), as it does in oocytes (Fig 1) There is no proof that the extremely low mouse α β ratios seen in eggs and morulae are significantly different from the low ratios seen in other stages Rabbit globin mRNA also yields a low ratio of α β globin when translated in unfertilised or newly fertilised eggs, on the other hand, this ratio changes progressively, in the absence of injected haemin, to an almost equal synthesis of α and β globin as the embryos develop (Table 1) The ratio of α β globin synthesis approaches one, when tested in embryos at

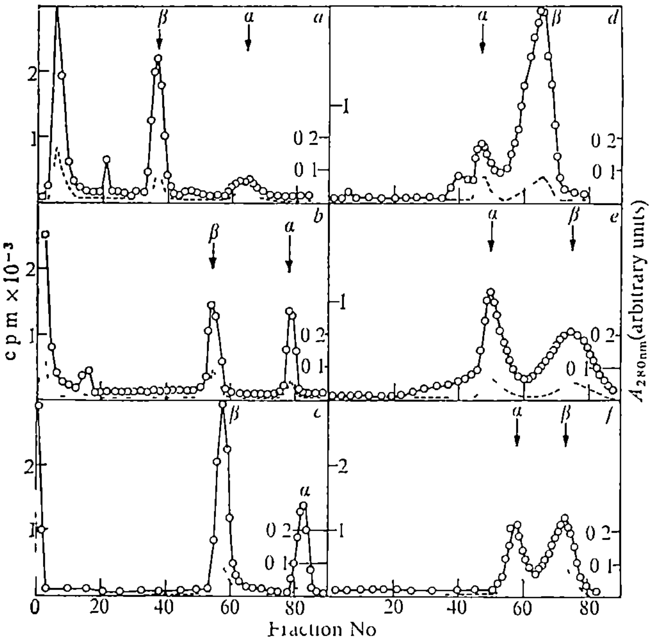

Fig 1 Effect of haemin on globin synthesis in oocytes injected with saturating and subsaturating amounts of rabbit and mouse globin messengers A stock of 16 mM haemin was prepared by the method of Zucker and Schulman[10] except that the final solution contained 90% (v/v) ethylene glycol Oocytes were injected with mRNA dissolved in saline[1], the latter containing haemin (1 6 mM) where indicated the intracellular concentration of haemin would have been about 80 μM Globin was prepared from whole oocytes and was fractionated by carboxymethyl cellulose chromatography[2] O——O, c p m per fraction, - - - -, absorbance at 280 nm of added marker α and β globin chains *a*, globin synthesis (α $\beta=17\%$) in oocytes injected with mouse mRNA (12 ng per cell), *b*, the effect of haemin (α $\beta=84\%$) on synthesis directed by such subsaturating amounts of mouse mRNA, *c*, the effect (α $\beta=46\%$) of haemin on saturating amounts (95 ng per cell) of mouse mRNA, *d* (α $\beta=12\%$) and *e* (α $\beta=84\%$) (+haemin) show analogous experiments with subsaturating rabbit mRNA (24 ng per cell), while *f* shows globin synthesis (α $\beta=55\%$) with saturating rabbit mRNA (48 ng per cell) plus haemin The α β globin synthesis ratio (α $\beta=20\%$) in oocytes injected with saturating messenger and no haemin is similar to that seen in oocytes injected with subsaturating mRNA Oocytes injected with buffer containing no RNA synthesise, no globin like species

Table 1 Ratio of α β-globin synthesis in mRNA-injected oocytes and eggs

Hours after fertilisation	Oocyte (ovarian)	Oocyte (matured)	Unfertilised egg 0	Morula 4	Blastula 8	Gastula 14	Neurula 20	Post-neurula 26	Early tail bud 44	Tail bud 50	Heart beat 54	Swimming tadpole 72	Stage 45 180
Mouse globin mRNA	20	—	3	5	24	10	10	20	14	15	11	11	—
Rabbit globin mRNA	17	20	19	22	67	59	60	68	66	—	—	54	54

Figures show ^{3}H-α-globin radioactivity as a percentage of ^{3}H-β-globin synthesis Oocytes and eggs were injected with mouse (12 ng per cell) or rabbit (7–20 ng per cell) globin mRNA[1 5] After the appropriate time, cells were labelled with ^{3}H-histidine by incubation (oocytes) or injection (embryos) Embryo homogenates were treated with acid acetone, and the resulting globin was chromatographed on a carboxymethylcellulose column[2] Matured oocytes were obtained by progesterone treatment (10 μg ml^{-1}) of immature oocytes

and beyond the neurula stage (20 h after fertilisation, Table 1)

Haemin can restore the efficient translation of rabbit and mouse α-globin mRNA in oocytes, but unlike the *Xenopus* embryo factor, it is equally effective with mouse and rabbit globin mRNA (Fig 1) For this reason the *Xenopus* factor is probably not itself haemin, though it may operate by part of the same mechanism since haemin and the embryo factor seem not to have an additive effect on rabbit globin synthesis in neurulae

Some information is available as to why less α globin is made than β globin For example this effect does not result from differential breakdown of completed α chains because α and β peptides are present in the usual 1 5 ratio in the polysomes of mRNA-injected oocytes[7] It also cannot be explained in a simple way by destruction of α rather than β mRNA because haemin increases the level of α-globin synthesis even when injected as long as 48 h after the mRNA (Table 2) It seems likely therefore that the effect is a result of a true translation control phenomenon

Two explanations of the haemin effect seem plausible One is that haemin raises the efficiency of initiation of α but not β-globin mRNA Alternatively haemin might remove some restriction to ribosome transit[7] located about half way along the α-globin mRNP particle Neither model necessitates a requirement for specific factors in the translation of specific messages and both models can be used to describe the agent enhancing rabbit α-globin synthesis during development Since haemin and the developmental factor differ in their effects on mouse globin synthesis, however, their mechanism of action cannot be identical The significance of these two regulatory phenomena is not clear In man, genetic deficiencies in haem synthesis are commonly associated with low globin synthesis and a low (0 71) ratio of α β-globin synthesis[8 9] Haem may therefore be involved in α-globin mRNA translation in normal reticulocytes The principal importance of the embryo factor, whose developmental significance is unknown, is that it indicates the possible existence in cells of a wide range of natural substances which may have very selective effects on message translation, the purification of those may help to reveal the kinds of mechanism by which translation can be controlled

Table 2 Effect of haemin when supplied after the initial injection of globin mRNA

Haemin injection (h after message)	Period of labelling (h after message)	α β (as a %)
No haemin	1–16	17
0 15	1–16	93
Simultaneous	1–16	84
No haemin	48–64	25
Simultaneous	48–64	83
48	48–64	57

Oocytes were injected with mouse globin mRNA (12 ng per cell) and were then injected with saline[1] or haemin The cells were labelled with ^{3}H-histidine Globin was prepared and fractionated[2] A further experiment was carried out by labelling oocytes for 5–18 h, with similar results to the 48–64 h experiments shown, except in the case of 'no haemin' samples About 30 ml of a 1 6 mM solution of haemin was injected per oocyte so as to give an intracellular concentration of about 80 mM

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Distinct haematological disorder with deletion of long arm of No. 5 chromosome

So far, the only specific chromosome abnormality in haematological disorders is the Ph1-chromosome Chromosome abnormalities have been described in so-called idiopathic sideroblastic or refractory anaemias[1] but a constant cytogenetic abnormality associated with a defined clinical syndrome has not been reported

Out of a series of patients with refractory anaemia or anaemia of-ill determined origin, three patients, a 46-yr-old male, and two females respectively 40 and 75 yr of age, were found to present a clinically very similar haematological disorder and an apparently identical chromosomal anomaly in the bone marrow

The haematological disorder is characterised by longstanding aregenerative slightly macrocytic anaemia with haemoglobin values ranging between 6 5 and 10 g dl, low to normal leukocyte count (3,000–4,000) and normal to elevated platelets (300,000–500,000) The bone marrow showed erythrocytic hypoplasia with evidence of disordered maturation and dyserythropoiesis, normal myeloid series but nevertheless moderate excess of blast cells (up to 15%), and absence of lobulation of the megakaryocytic nucleus Patients with a similar haematological syndrome have been described in the past[2] but no cytogenetic investigation was performed

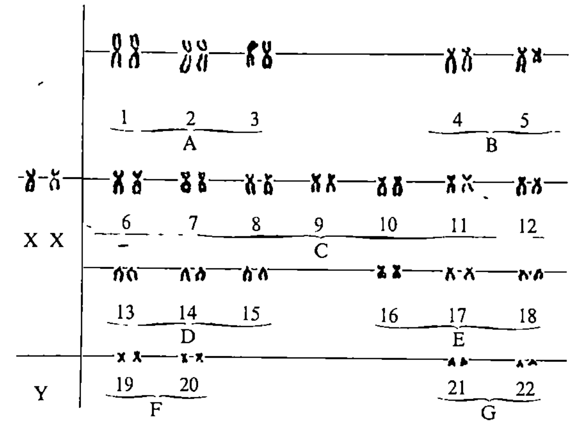

Fig 1 Routine karyotype (orceine) from a 48-h bone marrow culture of one of the patients

The chromosomal anomaly is a deletion of approximately two-thirds of the long arm of a chromosome No 5, without translocation of the deleted segment (Figs 1 and 2), present in 75–90% of the bone marrow cells cultured without PHA for 24–48 h No single abnormal cell has so far been found in peripheral blood cultured with PHA and in skin fibroblasts (Table 1), no metaphases were obtained from lymphocyte cultures without PHA The deletion seems to be interstitial and the following bands may be involved 5q 12–5, 5q 21–3 The association of this chromosomal anomaly with a defined haematological disorder may represent a newly recognised syndrome To our knowledge one case has been reported with similar clinical course and chromosome anomaly, but the B chromosome involved was not identified[3]

The absence of the deleted chromosome in the lymphocytes of the peripheral blood cultured with PHA, and in skin fibroblasts of our phenotypically normal patients indicates that the anomaly is an acquired one, occurring originally in a bone marrow stem cell, followed by clonal evolution and gradual replacement of an important part of the bone marrow

As far as can be concluded from the cytogenetic investigations, the abnormal cells are confined to the bone marrow It is possible that the stem cell with the abnormal chromosome is capable of erythroid, myeloid and megakaryocytic differentiation, but at present only morphological arguments can be found in favour of this hypothesis an erythroid hypoplasia, megakaryocytic nuclear anomaly, and a slightly reduced number of granulocytes with moderate blast excess in the marrow This situation could be very similar to that found in Ph1-positive disease Here also the chromosomally abnormal stem cell is capable of myelocytic, erythroid or megakaryocytic differentiation, and the extent of abnormal proliferation may be different for each cell line, for example, although the myeloid series is most often affected, polycythemia or thrombocytosis as well as anaemia or thrombopaenia can be the predominant haematological features So far there is no explanation for the variation in the haematological features of the Ph1-positive conditions Examination of a larger group of patients presenting with the 5q syndrome will be required before the extent of possible variability in the haematological features of this syndrome can be assessed

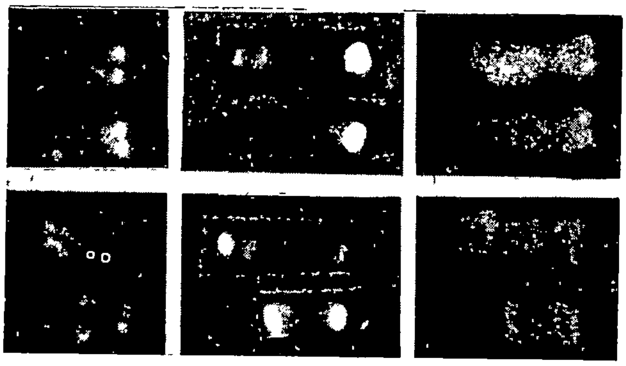

Fig 2 The B chromosomes of the three patients (T banding) showing that the chromosome involved is a No 5, of which most of the long arm is missing

Table 1 Summary of the cytogenetic investigations

Patients and tissues examined		No of cells with deletion 5q (46 cells only) Present	Absent	Undertermined
M G	(1) Marrow 48 h 1971	5	—	13
(♀ 40 yr)	(2) Marrow 48 h January, 1974	17	1	3
	(3) Marrow 48 h February, 1974	94	7	7
	(4) Skin Fibroblasts	—	30	—
E M	(1) Marrow 48 h September, 1973	98	18	8
(♀, 75 yr)	(2) Marrow 48 h March, 1974	87	15	15
	(3) Blood + PHA,, 72 h	—	50	—
	(4) Blood + PHA, 72 h	—	50	—
B A	(1) Marrow 48 h February, 1974	67	29	7
(♂, 46 yr)	(2) Blood + PHA, 72 h February, 1974	—	22	—

* No growth was observed in peripheral blood cultured without PHA

Finally it is to be noted that according to Price *et al*[4] one of the haemoglobin structural genes may be located on the long arm of a B chromosome If their findings are confirmed, and if the B chromosome turns out to be a No 5, a possible relationship between the deleted chromosome and the anaemia must be considered

More details about these patients will be published in a forthcoming paper H V D B , was supported by a grant from FWGO and J -J C and G D , by NFWO

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Cytophotometric factors causing apparent differences between Feulgen DNA contents of different leukocyte types

A CENTRAL issue in the study of both metazoan differentiation and the control of cellular activity is the validity or otherwise of the hypothesis[1,2] that nonreplicating nuclei of a given higher organism contain a constant amount of DNA per haploid set of chromosomes Differential elimination or increase of DNA has been demonstrated in dipteran polytene chromosomes[3], and in the oocytes of *Acheta* and amphibia[4], but apart from these possibly special cases the most studied apparent exception to the constancy rule is probably provided by mammalian leukocytes[5–11] Most workers in this field have found the apparent DNA content of the relatively large nuclei of monocytes, as assessed by Feulgen cytophotometry, to be between 5% and 20% higher than that of the smaller, more darkly stained nuclei of lymphocytes and polymorphonuclear leukocytes The consensus of opinion seems to be that the measured differences are probably due to nonstoichiometry of staining rather than to genuine differences in the amounts of DNA present But work published to date has in general been performed without certain technical refinements which have recently become available, and we have now investigated the possibility that systematic cytophotometric errors may have been responsible for at least some of the reported differences between cell types

Air-dried smears of normal adult human blood were fixed in methanol–formalin–acetic acid (80 10 5) for 1 h at room temperature, washed in running tap water for 60 min, hydrolysed in 5 N HCl at 25° C for 80 min (found in pilot experiments to give maximum staining intensity), rinsed in distilled water, stained 40 min in fresh Schiff's reagent (prepared by the de Tomasi method[12]), rinsed in distilled water, dehydrated in a series of alcohols and mounted in Polymount Cytophotometry at λ_{max} was performed using a Vickers M86 scanning microdensitometer with a 1 3 NA ×100 apochromatic objective The achromatic microscope condenser was used dry at full aperture The field illuminated was limited by a field stop to reduce glare, each nucleus was measured both with and without electronic compensation[13] for the measured residual glare, which was 4% in a series of experiments with a 40 µm diameter illuminated field, and 6% in a series with a larger illuminated area Two sizes of flying spot, respectively 0 48 µm and 0 95 µm diameter in the object plane, were used to permit correction for residual distribution error[14] The monochromator entrance slit was opened as far as possible to increase the signal/noise ratio, it could be shown (methods and results to be published) that chromatic error due to the relatively wide monochromator bandwidth (about 50 nm at total cutoff) was negligible under the stated conditions At least 10 measurements, each accompanied by a blank scan of empty background, were made of each cell under each set of conditions At least eight nuclei of each cell type studied (lymphocytes, monocytes and polymorphs) were measured on a single stained slide in each of two experimental series

Both series gave essentially similar results, those of the series with 4% glare being given in Table 1 It will be seen that with glare and residual distribution error uncorrected, the mean integrated absorbance (that is, apparent Feulgen DNA content) of monocytes was significantly higher than that of lymphocytes, which was in turn significantly higher than that of polymorphs When glare but not residual distribution error was corrected the difference between monocytes and lymphocytes disappeared, but both had significantly higher apparent Feulgen DNA contents than did polymorphs With correction for residual distribution error but not glare, lymphocytes and polymorphs had similar apparent Feulgen DNA contents, which were, however, lower than that of monocytes No significant difference between the three cell types was found if glare and residual distribution error were both corrected

The results suggest that in our uncorrected measurements glare is largely responsible for lymphocytes having a lower apparent Feulgen DNA content than monocytes, and that residual distribution error together with glare accounts for the still lower apparent Feulgen DNA content of polymorphs This interpretation is consistent with the morphological features of the three types of cell nucleus, since stained lymphocyte nuclei have a higher mean absorbance than those of monocytes (and are therefore more liable to be erroneously measured in the presence of glare), while polymorph nuclei have both a high mean absorbance and a less uniform distribution of chromatin than either monocytes or lymphocytes

We believe that uncorrected glare and distribution errors account for at least some of the previously reported apparent differences in the Feulgen DNA contents of different types of leukocyte, and that there is therefore no longer any compelling reason to postulate either genuine differences in the amounts of DNA present, or nonquantitative staining of DNA by the Feulgen method

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Table 1 Feulgen-DNA content of different types of leukocyte

	Integrated absorbance at λ_{max}			
	Uncorrected	Corrected for residual glare	Corrected for residual distribution error	Corrected for both residual glare and distribution error
Lymphocytes (L) $N=9$	19 24±0 25	21 67±0 15	19 46±0 28	21 89±0 21
Polymorphs (P) $N=9$	18 54±0 19	20 98±0 11	18 84±0 21	21 62±0 10
Monocytes (M) $N=8$	20 18±0 23	22 03±0 17	20 18±0 21	22 06±0 23
	L<M, t=2 76‡ L>P, t=2 23‡ P<M, t=5 50§	L<M, t=1 59* L>P, t=3 71§ P<M, t=5 19§	L<M, t=2 06† L>P, t=1 78* P<M, t=4 51§	L<M, t=0 55* L>P, t=1 16* P<M, t=1 75*

* Not significant
† P<0 06
‡ P<0 05
§ P<0 01
λ max was about 560 nm Results are given as mean integrated absorbance (in µm² units) ± s e

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C-type viral particles in a urinary bladder neoplasm induced by *Schistosoma haematobium*

NEOPLASMS may be induced in nonhuman primates after infection with *Schistosoma haematobium* (refs 1 and 2 and unpublished results of R E K, A W Cheever, B J Myers, S W Young and J A Moore) After various times, masses develop within the urinary bladders of the hosts, ranging from hyperplasia to papillary carcinoma as determined histopathologically Although the basic mechanism of tumour induction in such cases is not known, the many factors involved could include stimulation of endogenous virus infections It is therefore interesting that we have now found C-type viral particles in one out of four papillary carcinomas of capuchin (*Cebus* sp) monkeys experimentally infected with *S haematobium* None were seen in two infected animals that developed only hyperplasia and squamous metaplasia Previous examination of normal bladder tissue has failed to demonstrate similar C-type viruses For electron microscopy and infection of the animals with *S haematobium* we used published methods[1,3,4] The bladders were taken from monkeys previously infected with *S haematobium* (Table 1) Histological evaluation based on examination of biopsy samples is also provided (Table 1)

Table 1 Histological evaluation of bladders of capuchin monkeys infected with *S haematobium*†

Animal No /sex	No of cercariae inoculated	Duration of infection (weeks)	Egg passage* Faeces	Urine	
CA 23 M	2,000	113	+	++	Hyperplasia
CA 62 F	2,000	109	0	+	Slight hyperplasia, and squamous metaplasia
CA 64 M	1,000	109	+	0	Papillary carcinoma
CA 67 M	2,000	109	++	+	Papillary carcinoma and squamous metaplasia
CA 68 F	2,000	109	+	+	Papillary carcinoma and squamous metaplasia
CA 70 M	1,000	111	+	+++	Papillary carcinoma and squamous metaplasia

* +, ++, +++, Indicate few, moderate numbers, or many eggs in samples 1–3 weeks before indicated duration of infection

† Carried out by Dr A W Cheever, National Institute of Allergy and Infectious Diseases.

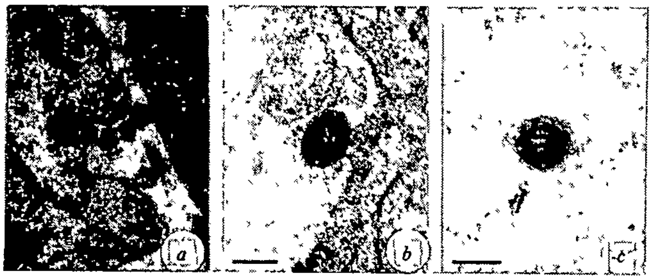

Fig. 1 *a*, Neoplastic transitional epithelium from urinary bladder of monkey No 70 with typical mature C-type viral particles in extracellular spaces *b*, C-type particle budding from plasma membrane *c*, Immature C-type particles in extracellular space (Scale 100 nm.)

Figure 1 shows electron microscopic evidence of C-type viruses at various stages of maturation associated with transitional epithelial cells in the bladder carcinoma of capuchin No 70 This tissue was the same as that examined by light microscopy Viral particles were readily observable throughout the transitional epithelial areas, but were absent in the loose connective and smooth muscle layers It should be emphasised that although one of the six monkeys examined was positive, this was the animal with moderate atypia As Table 1 shows, this animal was also passing the highest number of eggs in its urine, an indication of involvement of the bladder Control tissue, that is bladders of animals not infected with *Schistosoma* as well as those with minimal or minor involvement of the bladder, has so far failed to disclose the presence of C-type particles

No definitive conclusions concerning the aetiology of bladder cancer can be drawn from these observations, but they do offer an intriguing approach for further study Bladder cancers of nonhuman primates are extremely rare[5], and while coincidence cannot be ruled out, several other considerations must be examined It has been suggested that all primates, like some other animal species, contain endogenous C-type particles[6,7] Is the schistosome the cause or is it the triggering mechanism? On the other hand, is this tumour development due to an exogenous agent, which is carried by the parasite?

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Ethnicity is a significant factor in the epidemiology of rubella and Hodgkin's disease

SOME diseases of humans have an incidence which varies with ethnicity Epidemiologic patterns are seen for example in nasopharyngeal carcinoma[1], carcinoma of the liver[2] and Hodgkin's disease[3] There are quite marked differences in HL-A antigens between some ethnic groups, in particular, some antigens commonly found in caucasoid racial groups are infrequent in negroid groups and absent in mongoloid groups[4]

Our recent study (unpublished) of 61 caucasians (28 males) with congenital rubella revealed an increased incidence of HL-A antigens 1 and 8 (HL-A1 $\chi^2 = 9\ 57$, P corrected for 25 comparisons (P^c) $< 0\ 05$, HL-A8 $\chi^2=4\ 40$, $P^c < 0\ 89$), because of the very high occurrence of these antigens in the female subjects (HL-A1 $\chi^2 = 14\ 32$, $P^c < 0\ 003$, HL-A8 $\chi^2 = 9\ 61$, $P^c < 0\ 048$) Although the incidence of antigens 1 and 8 was not raised in the male subjects, those males who possessed these antigens were clinically more severely affected by the disease ($\chi^2 = 4\ 17$, $P < 0\ 04$)

We can now show that ethnic variations in the frequency of HL-A antigens 1 and 8 influence the epidemiology of rubella, and that striking similarities are found in the epidemiology of Hodgkin's disease These similarities support the suggestion that Hodgkin's disease has an infectious aetiological component but do not necessarily relate it to rubella

In order to determine whether the frequency of HL-A antigens 1 and 8 is relevant to rubella epidemiology throughout the world, we correlated (Fig 1) the percentage of young adults with serological evidence of previous rubella infection (that is, % seropositive) with the gene frequencies of HL-A1, HL-A8 and the mean gene frequency of HL-A1 and 8 for that ethnic composition (These were calculated from the Joint Report of the Fifth Histocompatability Workshop, 1972 (ref 4) It was sometimes necessary to calculate a mean gene frequency using the percentage of different ethnic groups in a particular population[5])

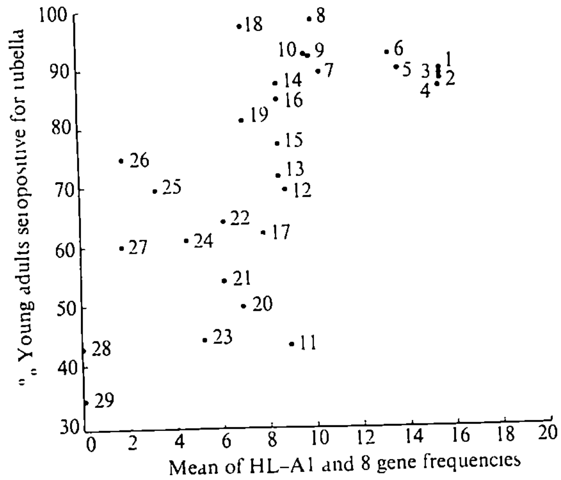

Fig 1 Geographical correlation between the mean of HL-A1 and 8 gene frequencies and seropositivity to rubella in young adults (aged approximately 20-29) HL-A gene frequencies were taken from ref 4 except for localities 4, 5, 6, 7 and 26, where refs 19, 21, 23, 25 and 34 were used respectively Correlation coefficient (r) = 0 68, t test on r (t_r) = 4 78, $P <$ 0 001 Localities indicated, together with references for the source of the rubella serology data, are as follows (1)=England[15], (2)=Australia[16], (3)=United States, Houston[17], caucasian, (4)=Sweden, Stockholm[18], (5)=Holland[20], (6)=Germany, West Berlin[22], (7)=Soviet Union, Moscow[24], (8)=Egypt[26], Arabs, (9)=Tunisia[27], (10)=Iran[28], (11)=Puerto Rico[29], (12)=Bermuda[30], (13)=Peru, caucasian[9], (14)=France, Lyons[17], (15)=Brazil, Sao Paulo[31], (16)=Argentina[9], (17)=Hawaii, all races[32], (18)=Soviet Union, Armenia[24], (19)=India[33], (20)=Panama[9], (21)=Surinam[20], (22)=Costa Rica[29], (23)=Trinidad[9,17], (24)=Jamaica[9 17], (25)=Peru, Indians and Mestizos[9], (26)=Singapore[17], (27)=Malaysia, Kuala Lumpur[7], (28)=Hawaii, Japanese[32], (29)=Japan, rural[17] In localities 9, 11, 12, 15, 16, 20, 22, 23, 26 and 27 ethnic composition was calculated from ref 5 as the exact ethnic composition of the population tested for rubella was not stated

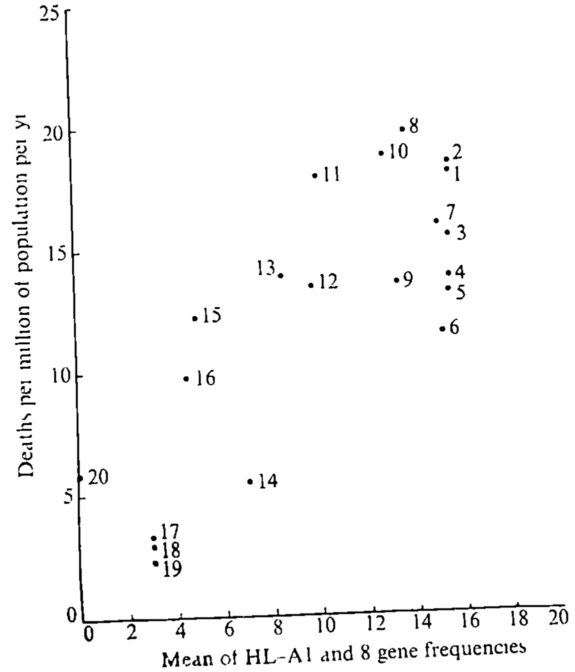

Fig 2 Geographical correlation between the mean of HL-A1 and 8 gene frequencies HL-A gene frequencies from ref 4 except where second references (19, 21, 23, 37) are cited, and deaths from Hodgkin's disease (all ages) per million of population per yr Correlation coefficient (r)=0 79, t test on r (t_r)= 5 47, $P <$ 0 001 Localities indicated in Fig 2, together with references for the source of the Hodgkin's disease data, are as follows (1)=United States, caucasian[3], (2)=England and Wales[35], (3)=Norway[3,19],(4)=Finland[3 19],(5)=Sweden[3,19],(6)=Australia[3], (7)=Canada[3], (8)=Holland[3,21], (9)=Germany[3,23], (10)=Denmark[3,19], (11)=Italy[3], (12)=India, Bombay, Parsi[35], (13)=France[3], (14)=India, Bombay, non-Parsi[35], (15)=United States, negro[36,37], (16)=Jamaica[38], (17)=Uganda[39], (18)=Kenya[40], (19)=Nigeria[41], (20)=Japan[3]

Linear regression analyses on the data for HL-A1 gave a correlation coefficient (r) of 0 72, t test on r (t_r) was 5 31 and the probability (P) was $<$ 0 001 For HL-A8, r was 0 61, t_r was 3 94, $P <$ 0 001, and for the mean gene frequency of HL-A1 and 8, r was 0 68, t_r was 4 78, $P <$ 0 001 These high positive correlations indicate that the frequencies of HL-A1 and 8 are an important factor in the worldwide epidemiology of rubella

By studying an epidemic of rubella, Hattis *et al*[6] have shown that there is a small number of infected individuals who have a high potential for spreading the virus ('spreaders') but that the majority of infected individuals have only minimal potential for spread We postulate that these spreaders who infect most susceptibles with whom they come in contact are those possessing the HL-A1, 8 haplotype The mean gene frequency of HL-A1 and 8 in a population would then be related to the number of potential spreaders, and consequently the extent and rate of transmission of virus in that population.

In the past, inexplicable differences in the mode of spread of rubella virus in various populations have been recorded. Statements concerning the spread in Hawaii[6], Malaysia[7], Taiwan[8], Jamaica and Trinidad[9], where the HL-A1 and 8 gene frequencies are low, may be related to the present hypothesis In all these areas there are fewer epidemics of rubella than expected, and in some the virus fails to become endemic in spite of high population densities This would seem to be due to the low HL-A1 and 8 gene frequencies causing sparser distribution of spreaders in a population which is genetically less susceptible to infection Although the occurrence of epidemics in these communities will also depend on the interaction of other factors such as opportunities for entry of the virus, the density of the population, and the level of herd immunity at the time of viral introduction, we propose that the most important factor is the frequency of the genes for HL-A1 and 8

Like that of rubella, the incidence of Hodgkin's disease varies in different ethnic groups, an infectious aetiological agent has been suggested, since the disease occurs in clusters[10], and a single contact can apparently give rise to multiple cases[11] Various studies in caucasians with Hodgkin's disease have revealed raised levels of several HL-A antigens[12], such as HL-A1 and 8, W5 and W18, all of which are absent from the Japanese[4], who have a later onset and slower rise of incidence of the disease[13] There is a significant increase in the incidence in Japanese moving to the United States In the United States, 71% of Japanese over the age of 50 are recent immigrants and in this older group the death rate from Hodgkin's disease rises sharply to, and even slightly above, the United States caucasian level[13] This is comparable to a group of non-immune individuals moving from a situation where an infectious disease is of low incidence and they have a low chance of contacting it, to an environment where it is more common and they have a much higher chance of contacting it, in this case the group moves from a population with low (or absent) frequencies of HL-A1 and 8 (Japan) to a population where these antigens are much more frequent (United States)

Table 1 Correlation of % mean gene frequencies of various HL-A antigens in 20 populations with deaths from Hodgkin's disease*

Antigen	*r*	t_r	*P*
HL-A1	0 79	5 47	<0 001
HL-A8	0 75	4 81	<0 001
HL-A1, 8	0 79	5 47	<0 001
HL-A3, 7	0 69	4 04	<0 001†
HL-A2, 12	0 39	1 79	Not significant
HL-A2, 7	0 54	2 72	,, ,,
W5	0 31	1 41	,, ,,
W18	0 39	1 48	,, ,,

* Deaths at all ages per million in that population per yr

† Populations which are predominantly caucasoid have high levels of HL-A antigens 3 and 7, and these antigens were more frequent (total % gene frequency 240) in our 20 test populations than HL-A antigens 1 and 8 (total % gene frequency 200) Thus the more significant association of HL-A antigens 1 and 8 ($r = 0\ 79$) with the incidence of Hodgkin's disease (compare 3, 7 $r = 0\ 69$) indicates that this relationship with HL-A1 and 8 is not simply due to a caucasoid trait not associated with HL-A

In order to determine whether HL-A1 and 8 played a part in the epidemiology of Hodgkin's disease similar to that found in rubella, data on the incidence of Hodgkin's disease in other ethnic groups were correlated with the mean gene frequency of HL-A1 and 8 (Fig 2) Linear regression analysis on the data for HL-A1 and HL-A8 produced high correlation coefficients (Table 1) The correlations with W5 and W18 were not significant We note that the high level of correlation with HL-A1 and 8 does not preclude association of Hodgkin's disease with some of the other HL-A antigens which also vary with ethnicity Populations which are predominantly caucasian have high levels of HL-A antigens 3 and 7 but the more significant association of HL-A1 and 8 with incidence of Hodgkin's disease indicated that this correlation with HL-A1, 8 was not simply due to a non-HL-A associated caucasoid trait

These similarities in the epidemiology of Hodgkin's disease and rubella support the concept that Hodgkin's disease has an infectious component, with the racial distribution of HL-A antigens controlling the degree of susceptibility of the population and the number of spreaders[11] Possibly there are other diseases of uncertain aetiology where an infectious agent has been implicated and where the incidence varies with ethnicity (for example multiple sclerosis[14]) in which there is a similar epidemiological pattern

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IgD on cell membranes of human lymphoid cell lines with multiple immunoglobulin classes

IMMUNOGLOBULIN D (IgD) is present in minute amounts in the serum and the biological role of this immunoglobulin class is unknown[1]. The finding that a high proportion of lymphocytes bear IgD on their surface membrane[2] has triggered renewed interest in this molecule.

IgD constitutes only 0.2% of serum immunoglobulins, but approximately 20% of circulating 'bursal' (B) lymphocytes bear surface IgD (ref. 2). In the newborn, serum IgD is usually undetectable and IgD seems to be the predominant class of surface Ig found on B lymphocytes (approximately 60%)[3]. These findings suggest that cell-associated IgD has an important biological function. Although cell surface Ig is normally restricted to a single class, individual lymphocytes with surface IgD can bear immunoglobulins of other classes as well. Thus, IgM has been found together with IgD in the membrane of peripheral blood lymphocytes of both adults and neonates[4,5] and on cells of patients with chronic lymphocytic leukaemia[6], in patients with Sjogren's syndrome the B lymphocytes seem to have IgG, IgA, and IgM on their surfaces in addition to IgD (ref. 7). Critical to the understanding of the significance of IgD, as well as the significance of multiple Ig classes on cell membranes, is whether these immunoglobulins are produced by the cells bearing them, or whether they represent cytophilic molecules or autoantibodies.

Here we report that IgD can be found on all the cells of a relatively high proportion of established human lymphoid cell lines grown in continuous *in vitro* culture. In addition, these IgD bearing cells were found to bear IgG, IgM and IgA. These findings provide direct evidence that lymphocytes produce the IgD found on their surface membranes and that individual lymphocytes can produce as many as four different surface Ig classes.

Cell surface Ig was demonstrated by staining the membranes of living cells with specific fluorochrome conjugated antisera. Of 25 cell lines tested, six were found to bear IgD (Table 1). Cell line 689 stained very brightly for IgD and all the cells in the culture were positive. In other cell lines the staining was less intense and although all the cells had surface IgD, the intensity of staining varied. Those cell lines found to bear δ (IgD) heavy (H) chains were examined for the presence of other H-chain classes in the membrane. In each case all the cells had surface γ (IgG), μ (IgM) and α (IgA) but not ε (IgE) H chains, and only one type of light (L) chain, K or λ. Several of the IgD negative cell lines were also found to bear more than one class of surface immunoglobulin. Because of this unexpected finding, multiple staining was confirmed using independently prepared purified antibodies, and precautions were taken to rule out nonspecific staining. The Fc receptor[10,11] found on B cells could bind spontaneously aggregated γ globulin present in the fluorochrome conjugated antisera. Removal of aggregates, however, by ultracentrifugation at 100,000*g* for 2 h did not affect the staining results. Non specific binding by the Fc portion of the fluoresceinated antibody to this receptor is unlikely as some fluorescent antibodies, for example, anti-IgE, did not stain the cells. This possibility was excluded when identical staining was obtained with the $F(ab')_2$ fragment of anti-IgG antibody, which lacks Fc determinants[8].

The state of these various H chains in the membrane was investigated. Membrane surface molecules can be aggregated by cross linkage with specific divalent antibodies[12]. When stained, these can be seen as fluorescent patches which are then actively transported to one pole of the cell to form a cap. Cells of cell line 698 were stained with fluorescein conjugated antibody to K L chain, washed, and incubated at 37° C for 1–3 h to allow capping to occur. Cells were then incubated with rhodamine-conjugated antiserum with specificity for an H-chain class. This second incubation was performed at 4° C in the presence of sodium azide (0.1%), conditions which prevent capping. Capping induced by antibody to the single L-chain type caused each H-chain determinant to cap along with the L chain. That is, the antiserum directed against each H chain stained only the cap. This indicates that δ, γ, μ and α H chains are each associated with an L chain on these cells and probably exist as discrete Ig molecules. Purified antibodies directed against H chains were less effective in producing caps. Anti-μ and anti-δ reagents induced caps in approximately 30% of cells of the 698 cell line. In these instances repeat staining with antibody to the same H chain stained only the cap and antibodies directed against each of the other H chain classes stained the cell diffusely. Thus μ and δ chains each capped independently of the other three H chains. This is similar to the finding that μ and δ chains cap independently of each other on peripheral blood lymphocytes[4,5]. Antibodies to γ and α chains stained the cells less intensely and did not produce distinct cap formation.

The cell lines are grown in continuous long term culture in the absence of exogenous human immunoglobulins. It was important to rule out the possibility that the observed staining was due to a few cells in the culture secreting IgD or other immunoglobulins, which then adhered to the remaining cells because of cytophilic properties. Human lymphoid cell lines can secrete more than one Ig class, but secreted IgD has not been found[13].

Cell line 698, which had the most intense staining for each of these surface Ig classes, was tested for immunoglobulin secretion. The cells were incubated with ^{14}C-leucine and the incubation medium then analysed for newly synthesised and secreted Ig by radioimmunoelectrophoresis[14]. The cells secreted only IgG-K molecules. This result is consistent with previous reports that membrane-bound Ig and secreted Ig are regulated by separate mechanisms[15].

These findings provide direct evidence that surface IgD is synthesised by the cell that bears it and is not merely adherent because of cytophilic properties[4,6]. Furthermore, it seems that individual lymphocytes have the capacity to produce four different surface Ig classes (IgD, IgG, IgA and IgM). The evidence of Litwin *et al.*[16] that lymphoid cell lines have both μ and γ chains on their surfaces is consistent with these findings. The significance of multiple Ig classes on single lymphocytes is not clear. Although it is tempting to speculate that a switch of surface $\delta \rightarrow \mu \rightarrow \gamma \rightarrow \alpha$ L chains occurs during the ontogeny of the lymphocyte[4], similar to the switch occurring fro μ to γ with secreted immunoglobulins[17], the distribution of multiple H chains found in these cell lines does not support such a hypothesis. No cells lines were found to bear δ or evenδ plus μ chains exclusively. Indeed, all the δ bearing lines also bore μ, γ and α chain. We expect that these different Ig classes will have identical L chains and will share identical H chain variable regions as reported for myelomas secreting two different Ig classes[17]. Each class may be involved in triggering a specific lymphocyte function. The predominance of IgD bearing cells in the neonatal period suggests that this class of molecules may be involved in the induction of immunologic tolerance[4]. The existence of cell lines in which every lymphocyte bears both IgD and multiple other Ig classes provides a powerful tool for the *in vitro* investigation of these questions.

In the normal adult, differentiation has resulted in restricted expression of surface Ig classes. Multiple H chain determinants, however, have been found on individual lymphocytes in some patients with chronic lymphatic leukaemia[18–20] and also in patients with Sjogren's syndrome, an autoimmune disease in which there is a spectrum of benign to malignant lymphoproliferation[7]. These abnormali-

Table 1 IgD bearing cell lines

Cell line	Source	Membrane immunoglobulin IgD	IgG	IgA	IgM	κ	λ
PG-B-1	Normal adult	+	−	+	+	−	+
MBK-1	Infect mono *	+	−	+	+	+	−
698	Lymphoma	+	+	+	+	+	−
Cat-B	Hypogammaglob *	+	+	+	+	+	−
Pt3L3	Xerod pigment*	+	+	+	+	+	−
Pt3L4	Xerod pigment*	+	+	+	+	+	−
Molt-4	Acute leukaemia	−	−	−	−	−	−
8866	Acute leukaemia	−	+	−	−	+	−
4265	Acute leukaemia	−	+	+	−	+	−
SB	Acute leukaemia	−	−	−	+	+	−
IM-1	Lymphoma	−	−	+	+	+	−
IM-9	Multiple myeloma	−	+	+	−	+	−
Raji	Burkitt lymphoma	−	−	−	+	+	−
EB-3	Burkitt lymphoma	−	−	−	+	+	−
Maku	Burkitt lymphoma	−	nt	nt	nt	+	−
BSO-B1	Burkitt lymphoma	−	+	+	+	+	−
BFK-B-1	Burkitt lymphoma	−	+	+	+	+	+
Kaplan	Infect mono	−	nt	nt	nt	+	−
MBK-B-1	Infect mono	−	nt	nt	nt	+	−
Fl-B-1	Foetal liver	−	−	+	+	−	+
Fl-B-4	Foetal liver	−	nt	nt	nt	+	−
Fl-B-6	Foetal liver	−	−	+	+	−	+
NC-37	Normal adult	−	−	−	+	+	−
PG-1	Normal adult	−	nt	nt	nt	+	−
GK-B-1	Normal adult	−	+	+	+	+	+

The cells in these cultures grow in suspension and are fed twice weekly with RPMI 1640 medium (NIH Media Unit) supplemented with 10% foetal calf serum (FCS) (Grand Island Biological NY) These cell lines have undergone a minimum of sixty subcultures Monospecific antiserums to IgG, IgA and IgM were prepared as previously described[2,7] using insoluble immunoabsorbents Special precautions were taken to ensure specificity of anti-IgD (refs 2 and 7) The antiserum was sequentially absorbed with Sepharose coupled to IgG, IgA, IgM, free L chains, and serum from a subject with low IgD levels No blocking occurred when the cells preincubated with non-fluorescein labelled anti-IgM or anti-IgG were tested for staining with fluorescein-conjugated anti-IgD When an antiserum specific for IgD (obtained by immunisation of a goat with IgD-λ myeloma protein) was absorbed with a different IgD preparation (IgD-κ) coupled to Sepharose, or with Sepharose-IgA-κ, only the IgD absorbed preparation had a markedly diminished staining capacity For capping experiments, purified antibody to IgD was prepared by absorption and elution from purified IgD-Sepharose[1] Antibody to κ chains was prepared using Sepharose-IgG-κ and antibody to λ chains prepared using Sepharose-IgE-λ Multiple H-chain staining was confirmed using independently prepared purified antibodies The antisera or antibodies were conjugated with fluorescein or tetra-methyl-rhodamine isothiocyanate[9] The fluorescein or rhodamine to protein (F/P) molar ratio of the reagents used ranged from 1 1 to 2 8 1 Viable cells were centrifuged and the pellet containing 2×10^6 cells incubated with 0 1 ml of undiluted fluorescein or rhodamine conjugated antiserum at 4° C for 30 min Sodium azide was added when it was necessary to prevent capping The cells were washed and suspended in medium (RPMI 1640 medium containing 5% FCS) on sides with vaseline-sealed coverslips The cells were examined by means of a Leitz Ortholux microscope with an Osram HBO 200 mercury arc lamp and a Leitz vertical illuminator

* Infectious mononucleosis, Hypogammaglobulinaemia, Xeroderma pigmentosum IgD bearing cell lines were tested for surface IgE and were found to be negative, nt, not tested

ties may represent dedifferentiation of the malignant or abnormal cells which permits the expression of multiple Ig classes in the membrane in a manner similar to that seen in human lymphoid cell lines On the basis of the findings in this report cytophilic molecules or autoantibodies need not be invoked to explain the finding of multiple Ig classes in lymphocyte membranes in these clinical states

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Marrow environment not required for differentiation of B lymphocytes

In birds, B lymphocytes differentiate from primitive progenitors in the Bursa of Fabricius, which is thought to provide a unique inductive stimulus, as the thymus in both birds and mammals is thought to provide a stimulus for the differentiation of T lymphocytes A bursal equivalent has not, however, been identified in mammals Two proposed sites are the bone marrow[1] and the gut-associated lymphoid tissue[2] We now have evidence, however, that the bone marrow is not an essential site for the differentiation of B lymphocytes in mammals

B lymphocytes differentiate from pluripotent stem cells in the bone marrow[3 4] These cells are probably identical or closely related to haemopoietic stem cells Specificity-restricted precursors of B cells (PB cells) are also found in bone marrow and seem to represent a stage intermediate between stem cells and functional B lymphocytes[5 7] PB cells have immunoglobulin (Ig) receptors but cannot initiate immune responses without further differentiation Thus, the differentiation pathway of B lymphocytes can be subdivided into at least two stages the differentiation of PB cells from stem cells, and the production of B cells from PB cells We have investigated the relative role of the bone marrow environment on these two stages

Table 1 CFU-S survival in femur from ^{89}Sr-treated donors

Time at 4° C (h)	Donor	Cells per femur (×10^5)	CFU-S per femur*	% Survival*
0	Control	180	3,700±530	13±6 0
	^{89}Sr-treated	110	490±210	
24	Control	220	1,800±550	10±3 4
	^{89}Sr-treated	130	180± 29	
48	Control	180	1,500±200	0 80±0 16
	^{89}Sr-treated	68	12± 1 7	

Mice (F_1 hybrids between C57BL/6 and C3H) received an intraperitoneal injection of 50 μCi ^{89}Sr Twenty-four hours later the femurs were removed from fifteen of these mice and from fifteen normal mice These femurs were placed in a Petri dish on filter paper moistened with phosphate-buffered saline and stored at 4° C At the times indicated ten femurs were taken from each group and used to prepare marrow cell suspensions Aliquots were injected into irradiated (950 rad) recipients to measure CFU-S

* Mean values ± one s e m

It is possible by injection of radioactive strontium (^{89}Sr), to make the marrow a hostile environment for differentiation[8,9] This isotope is taken up exclusively by bone When ^{89}Sr decays, it emits 1 5 MeV electrons and irradiates the bone marrow cavity with doses of radiation that inhibit proliferating cells[8] Because the energy of the radiation is insufficient to penetrate bone and irradiate other organs in the animal, it is possible by this means to irradiate specifically and continuously the entire bone marrow of an animal without affecting the integrity of other organs

We first estimated how rapidly cells in bone marrow were inactivated by an intraperitoneal injection of ^{89}Sr Twenty-four hours after injection, the intact femurs were taken from these mice and from normal mice and stored at 4° C After various times, the marrow was collected from these femurs and the number of haemopoietic stem cells per femur was determined by injection of the cells into irradiated recipients These stem cells form easily detectable colonies in the spleens of irradiated mice, and enumeration of the number of spleen colony-forming units (CFU-S) provides a quantitative estimate of the number of surviving haemopoietic stem cells[10,11] Because the radiation sensitivity of CFU-S and B cells is similar[12], the survival of CFU-S in ^{89}Sr-treated recipients should be similar to that of B cells and their precursors located in marrow These measurements (Table 1) suggest that CFU-S are killed exponentially with time and have a halflife of approximately 10 h in a marrow environment irradiated by ^{89}Sr In four other experiments in which CFU-S survival was measured 4 d after ^{89}Sr treatment, survival was always about 1% relative to untreated controls

On the basis of this short halflife for a known stem cell in ^{89}Sr-treated recipients, we attempted to measure the function of mature B cells, PB cells and lymphoid stem cells in mice with defective marrow Recipient mice received intraperitoneal injections of 50 μCi of ^{89}Sr Four days later, both these mice and untreated controls received whole body irradiation from a ^{137}Cs irradiator with doses ranging from 750 to 950 rad and were used to measure B cell, PB cell or stem cell activity as indicated below

To test for loss of B cell activity, each recipient received 2×10^7 spleen cells from normal donors, together with 5×10^8 sheep red blood cells (SRBC) Eight days later, they were killed and antibody-producing cells were counted per spleen using the Jerne plaque-forming cell (PFC) assay[13], to give a measure of B-cell activity in these recipients Table 2 shows that in three experiments, B-cell activity was not affected by pretreatment of recipients with ^{89}Sr, if anything, the response was greater in pretreated recipients than in controls

Table 2 Function of lymphoid and haemopoietic cells in ^{89}Sr-treated mice

Assay	Experiment no	Response in†		$\frac{^{89}\text{Sr-treated}}{\text{control}}\times 100$‡
		Control recipient	^{89}Sr-treated recipient	
B cell	1	703 PFC per spleen	987 PFC per spleen	140 (112–176)
	2	629 PFC per spleen	1,803 PFC per spleen	286 (185–442)
	3	1,504 PFC per spleen	1,196 PFC per spleen	79 (51–122)
PB cell	1	1,278 PFC per spleen	730 PFC per spleen	57 (34– 96)
	2	1,606 PFC per spleen	899 PFC per spleen	56 (31–102)
	3	6,792 PFC per spleen	1,993 PFC per spleen	29 (20–42)
Stem cell	1 (25)*	22,600 PFC per spleen	14,600 PFC per spleen	65 (38–109)
	2 (24)	25,300 PFC per spleen	11,100 PFC per spleen	44 (35–55)
	3 (33)	26,000 PFC per spleen	15,600 PFC per spleen	60 (41–88)
CFU-S in marrow	1 (25)	636 CFU-S per femur	35 CFU-S per femur	5 5 (4 8–6 2)
	2 (24)	860 CFU-S per femur	16 CFU-S per femur	1 9 (1 3–2 5)
	3 (33)	1,206 CFU-S per femur	52 CFU-S per femur	4 3 (2 8–5 8)
CFU-S in spleen	1 (25)	783 CFU-S per spleen	1,933 CFU-S per spleen	247 (197–297)
	2 (24)	1,500 CFU-S per spleen	2,800 CFU-S per spleen	187 (159–215)
	3 (33)	1,512 CFU-S per spleen	2,889 CFU-S per spleen	191 (149–233)

In experiments 1 and 2 the primary recipients received 750 rad whole body irradiation, in experiment 3, 900 rad

* The numbers in parentheses are the days after transplantation, that is, in experiment 1, the stem cell and CFU-S assays were done 25 d after transplantation of cells into the irradiated (950 rad) recipients

† The PFC responses are geometric means of the responses measured in individual spleens The CFU data are arithmetic means

‡ The values in parentheses are one standard error above and below the mean

To measure PB cell activity, bone marrow was separated by velocity sedimentation as described previously[5,14] Those cells sedimenting between 4 5 and 8 0 mm h^{-1} were pooled to provide a suspension enriched for PB cells and stem cells but nearly free of B cells[6] and 3×10^6 cells from the pool were injected, together with 5×10^7 thymus cells, into recipient mice prepared as above Seven days later the recipients were immunised with an intraperitoneal injection of 5×10^8 SRBC Eight days after immunisation, the PFC per spleen were measured for each mouse Lafleur *et al*[5] showed that under these conditions the PFC response is derived primarily from PB cells Table 2 shows that the differentiation of PB cells is not markedly suppressed in ^{89}Sr-treated recipients

The differentiation of B lymphocytes from cells more primitive than PB cells was also studied according to the method of Lafleur *et al*[6] Cells were separated and injected into recipients as described above Approximately 4 weeks later, recipients were given an intravenous injection of 5×10^7 thymus cells (to ensure that there was no defect in T cell function) and 5×10^8 SRBC Six days later, at the time of peak response, the PFC response was determined As with PB cells, there was a slight suppression in the PFC response in ^{89}Sr-treated recipients (Table 2)

Radioactive strontium has a halflife of 54 d and should therefore have suppressed the activity of cells in marrow throughout the time period of our experiments This suppression was confirmed by measurements of CFU-S in the femurs and spleens of the ^{89}Sr-treated recipients When mice were killed for measurement of PFC for the stem cell assay, their spleen and marrow cells were also injected into normal irradiated recipients to count the CFU-S CFU-S content in the femurs of ^{89}Sr-treated recipients was suppressed markedly compared with the control (Table 2) Also, as reported before[8], the spleens of such recipients had increased numbers of CFU-S These results show that cells such as CFU-S, which must proliferate to function, do not survive in the marrow during the time required for experiments such as ours

The CFU-S data clearly show that pretreatment of mice with ^{89}Sr effectively destroyed the bone marrow environment as a site for normal differentiation Nevertheless, the differentiation of PB and stem cells in such recipients was only slightly suppressed If this differentiation had to occur by proliferation of stem cells in the bone marrow environment, the suppression of activity should have been of the same order as seen for CFU-S in the marrow That such suppression was not observed is evidence that the marrow environment is not essential for the development of B lymphocytes

There are several possible explanations for the small suppression observed for the PB cells and stem cells assayed in ^{89}Sr-treated recipients Perhaps the most likely is that it is a result of the ability of many B cells to circulate through the lymphoid system[15] Because of this property, it is reasonable to expect that some B cells will be inactivated as they migrate through the bone marrow In support of this suggestion is our observation that the B cell activity in spleen decreases to approximately 30% of normal within 4 d after injection of 50 μCi of ^{89}Sr (unpublished data of R A P)

An alternative explanation of the suppression of PB and stem cell activity is that the differentiation of functional B cells requires a transient exposure to elements in bone marrow If the radiation sensitivity of B cell precursors is the same as for B cells and CFU-S, a suppression of 50% would be consistent with a 10-h exposure to the marrow environment This 10-h estimate is an upper limit and the data are consistent with there being no required residence time in marrow

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Oestrogen receptors and antagonism of steroid hormone action

NON-STEROIDAL anti-oestrogens antagonise the effects of oestrogen on uterine growth, vaginal cornification and ovulation[1], and their mechanism of action has been suggested to reside in their ability to compete for cytoplasmic oestrogen binding sites, or receptors, thereby reducing the formation of receptor oestrogen complexes[2–4] Since the formation of these complexes in oestrogen target tissues, with subsequent translocation to nuclear sites, is considered the primary event in oestrogen action[5,6], the reduction in their number would lead to decreased physiological responses to oestrogen This implies that the receptor antagonist complex should have a lower intrinsic biological activity than that of the receptor oestradiol complex, that is, the ability of the receptor antagonist complex to stimulate oestrogenic responses would be less than that of the receptor oestradiol complex Based on these assumptions, oestrogen antagonism should be observed after a single injection of oestrogen and antagonists, we have now found that this is not the case Thus, non-steroidal anti-oestrogens could not be acting as antagonists by competing initially for oestrogen receptors

Immature rats, 21–22 d old, were injected with 2 5 μg oestradiol, 100 μg nafoxidine hydrochloride (Upjohn 11,100A 1-(2-[P-(3,4-dihydro-6-methoxy-2-phenyl-1-naphthyl)phenoxy] pyrrolidine hydrochloride) or a combination of the two, and uterine dry weights were determined 24–48 h later The increases in uterine dry weight after a single injection of oestradiol, nafoxidine or oestradiol plus nafoxidine were identical 24 h after injection By 48 h nafoxidine or oestradiol plus nafoxidine was more effective than with oestradiol alone in stimulating uterine growth (Fig 1) Therefore, after

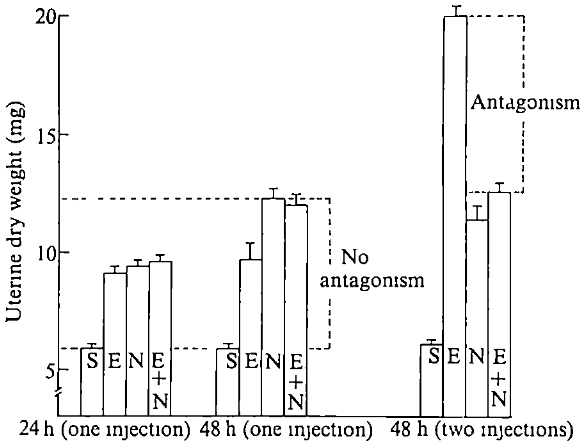

Fig 1 Agonistic and antagonistic effects of nafoxidine on uterine growth Immature female rats were injected with saline (S), 2 5 μg of oestradiol (E), 100 μg of nafoxidine (N) or oestradiol plus nafoxidine (E + N) according to the following design Two groups received one injection and uterine weights were determined 24 or 48 h later A third group received two injections 24 h apart and uterine weights were determined 24 h after the second injection Each value represents the mean ± s e m for three or four determinations with five or six animals per determination

a single injection of oestradiol plus nafoxidine there was no antagonism, indeed, nafoxidine clearly acted as an oestrogen The antagonistic properties of nafoxidine could be observed, however, when the compounds were administered at 24 h intervals and the uterine weights were determined after 48 h (Fig 1) Serial injections (between three and eight) at 24 h intervals have often been used routinely and it is well established that oestrogen antagonism can be observed under these conditions (refs 1 and 7–9 and Fig 1, two injections) Such routine methodology has resulted in the failure to detect a lack of antagonism after a single injection It should be noted that we have since used much smaller doses of antagonists than were injected in the work described with similar results, that is, smaller doses, 1–10 μg have both agonistic and antagonistic properties (to be published elsewhere)

We have proposed that anti-oestrogens antagonise oestrogen-induced uterine growth as a result of their failure to stimulate the replenishment of the cytoplasmic oestrogen receptor[10] That is, after cytoplasmic binding and nuclear translocation of receptor antagonist complexes, no additional receptor is provided to the cytoplasmic pool This failure to stimulate the replenishment of cytoplasmic receptor renders the uterus insensitive to subsequent injection of oestrogen and therefore antagonism can be observed

To test this hypothesis further and to establish the general nature of this phenomenon, we have examined the effects of two other oestrogen antagonists, CI-628 and (α-[4-pyrrolidineothoxy]phenyl-4-methoxy-α-nitrostilbene) and clomiphene (Triecthylamine, 2-(P-[-chloro-1,2diphenyvinyl]-phenoxy)-citrate) These compounds, as well as nafoxidine, were administered either alone or in combination with oestradiol, and the uterine weight and cytoplasmic oestrogen receptor content were determined The concentration of cytoplasmic receptor was determined by the ^{3}H-oestradiol exchange–charcoal adsorption method[10 11] which facilitates evaluation of total cytoplasmic receptor and avoids complications arising from assays that measure only free or unfilled receptor sites[12] Bound nafoxidine, CI-628 and clomiphene (our unpublished results) are freely exchangeable with ^{3}H-oestradiol in this assay procedure[10,11]

As Table 1 shows, all oestrogen antagonists examined were clearly uterotrophic and not antagonistic when given in combination with oestrogen Thus, CI-628 and clomiphene are similar to nafoxidine in this regard In addition, all three antagonists, alone or in combination with oestradiol, reduced the quantity of cytoplasmic receptor 24 h after treatment (Fig 2) Thus none of these antagonists could stimulate the replenishment of the cytoplasmic receptor, whereas oestradiol treatment resulted in a significant increase in receptors (Fig 2) Therefore, the ability of the uterus to respond to oestradiol 24 h after treatment with an antagonist would have been greatly reduced This was clearly the case with nafoxidine, after which subsequent oestradiol treatment induced no uterine growth (Fig 1) In contrast, the quantity of cytoplasmic receptor in animals pretreated with oestradiol was twice that of the controls and the uterus was very responsive to a second injection of oestrogen (Fig 2)[10] Thus these compounds are both agonistic and antagonistic The anti-oestrogens are agonists because they stimulate the metabolic and regulatory pathways that cause uterine growth They are also antagonists, however, because they fail to stimulate the replenishment of the cytoplasmic receptor and thus leave the uterus insensitive to oestrogen

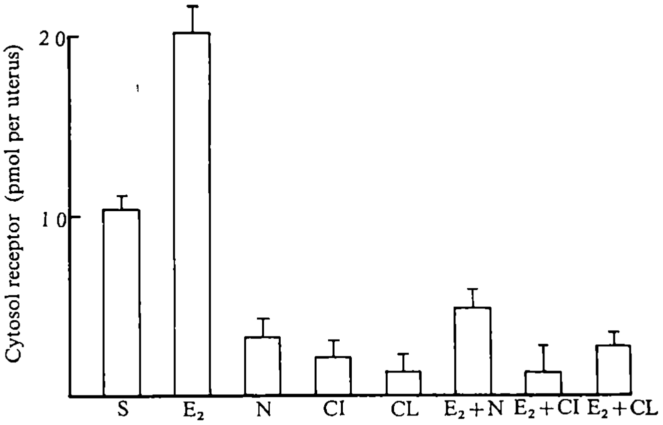

Fig 2 Effect of oestradiol and oestrogen antagonists on the quantity of cytoplasmic receptor 24 h after treatment Immature female rats were injected with 2 5 μg of oestradiol (E), 100 μg of nafoxidine (N), 0 5 mg of CI-628 (CI), 10 0 mg of clomiphene (CL) or a combination of oestradiol plus the antagonists The quantity of total cytoplasmic receptor was determined 24 h after treatment by the ^{3}H-oestradiol exchange–charcoal adsorption procedure (see text) Each value represents the mean of three or four experiments with five or six animals per experiment

Table 1 The effect of oestradiol and oestrogen antagonists on uterine growth after a single injection

Treatment	Uterine wet weight (mg) 24 h	Uterine wet weight (mg) 48 h
Saline	36 8 ± 1 5	40 5 ± 2 3
Oestradiol (2 5 μg)	53 4 ± 1 0	60 8 ± 2 7
Nafoxidine (100 μg)	54 2 ± 2 0	60 3 ± 4 2
CI-628 (500 μg)	51 2 ± 2 1	62 0 ± 3 6
Clomiphene (10 mg)	50 1 ± 2 8	69 0 ± 4 5
Oestradiol + nafoxidine (2 5 + 100 μg)	54 5 ± 1 9	63 2 ± 3 9
Oestradiol + CI-628 (2 5 + 500 μg)	52 9 ± 2 3	72 3 ± 5 7
Oestradiol + clomiphene (2 5 + 10 mg)	50 3 ± 2 8	74 0 ± 6 8

Immature female rats were injected with the compounds indicated and the wet weight of their uteri was determined 24 or 48 h later All values represent the mean ± the s e m

A further factor that might contribute to oestrogen antagonism is that anti-oestrogens might stimulate cellular hypertrophy, thereby increasing uterine weight, but fail to stimulate uterine hyperplasia In such a case the number of cells able to respond to subsequent oestrogen treatment would be reduced and this reduction would result in diminished utertrophic responses than when oestrogen alone was used To examine this possibility and to characterise further the growth induced by the anti-oestrogens, we have measured the quantity of DNA and protein in the uterus at various times after the administration of hormone or antagonist Both oestradiol and nafoxidine increased DNA content within 48 h, thus there was no apparent difference in their capacity to elicit uterine hyperplasia (Fig 3) Increased mitotic activity has also been observed to occur with either compound (unpublished results) The obvious difference between the uterotrophic responses elicited by the two compounds was the sustained stimulation of protein synthesis observed with nafoxidine (Fig 3) Apparently the long term uterotrophic effects of nafoxidine arise from the ability of the receptor antagonist complex to stimulate cellular hypertrophy These experiments indicate that oestrogen antagonism is not due to an inability of nafoxidine to stimulate cell growth In fact, it was more effective than oestradiol when uterotrophic responses were compared 72 h after treatment (Fig 3)

These studies are not sufficient to determine whether receptor replenishment is the only uterotrophic response not stimulated by oestrogen antagonists We feel, however, that most response pathways are stimulated since the increases in uterine protein, DNA and weight induced by the antagonists are equal to those induced by oestradiol Our findings are important in the interpretation of experiments in which oestrogen antagonists have been used to block ovulation, implantation and other oestrogen-stimulated responses In these systems oestrogen antagonists may stimulate oestrogenic responses just as in the uterus and, in a similar fashion, may not stimulate the replenishment of cytoplasmic receptor If this is true, the agonistic nature of the anti-oestrogens as well as their inability to promote the replenishment of the cytoplasmic receptor, and its subsequent binding and interaction with oestrogen, would become important considerations in the interpretation of such systems

Our investigation has important implications with respect to the mechanism of action of non-steroidal antagonists of oestrogenic hormones Theories and model systems designed to describe the action of oestrogen antagonists must include more than a simple competition for steroid binding sites It has been assumed that the dissociation constant, K_d, for the binding of antagonist to steroid receptors is related directly to its capacity to block steroid activity, that is, the K_d is that concentration of antagonists that will block or antagonise 50% of the maximal (physiological) biological response Assays for the action of oestrogen antagonists, however, usually have involved serial injections The marked depletion of cytoplasmic receptor under such conditions would invalidate the model because implicit in it is the assumption that receptor is present at normal levels during each period of treatment

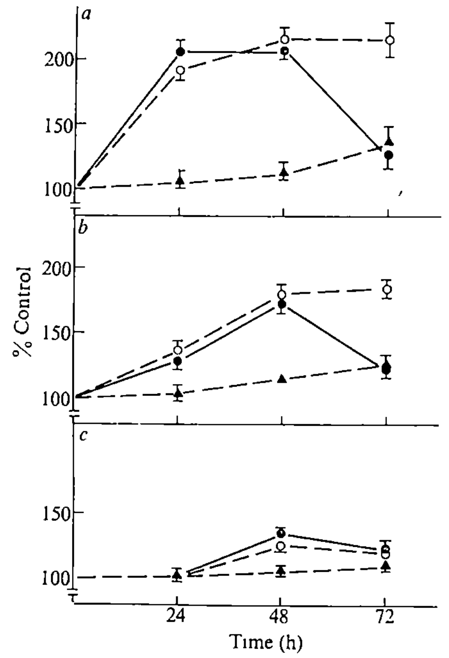

Fig 3 Effect of oestradiol and nafoxidine on uterine protein and DNA after a single injection Immature rats were injected with 2 5 µg oestradiol (●), 100 µg of nafoxidine (○) or saline (▲) and the wet weight, protein[13] and DNA[14] were determined 24, 48 and 72 h later The control values at the beginning of the experiment were wet weight, 32 5 ± 3 1 mg per uterus, protein, 4 5 ± 0 2 mg per uterus, DNA, 360 ± 12 µg per uterus Each value represents the mean of five or six determinations with three to six animals per determination *a*, Wet weight, *b*, protein, *c*, DNA

In conclusion, we believe that non-steroidal anti-oestrogens are antagonists not because they compete for cytoplasmic oestrogen receptor to produce a complex which is a poor stimulator of growth, but because they fail to stimulate the replenishment of the receptor Thus the uterus of the antagonist-treated animal contains relatively few cytoplasmic receptors and is insensitive to oestrogen, whereas the uterus of the oestrogen-treated animal contains many cytoplasmic receptors and is highly sensitive to oestrogen

This work was supported by grants from the National Institutes of Health and the American Cancer Society We thank Dr P W O'Connel, Upjohn Co , Dr Jerry Reel, Parke-Davis and Co and Mr A Richardson, jun , Merrell-National Laboratories for the nafoxidine, CI-628 and clomiphene We also thank L Hseuh for technical assistance

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reviews

Bringing the dead to life

The Mound People By P V Glob Pp 184+75 plates (Faber and Faber London, May 1974) £4 25

BARRY CUNLIFFE

ONCE more Professor Glob puts us in his debt Five years ago his best seller *The Bog People* was published in English, now we are presented with its sequel—*The Mound People*—a lively and enthralling account of the inhabitants of Denmark at one of the peaks of the country's cultural development in the second millennium BC In the first part of the book the author sets out to describe a selection of the remarkably preserved burials by which the Bronze Age inhabitants are known Having thus introduced them to us, down to the last detail of their clothing, he proceeds to reconstruct something of the social economic and religious background of the times

That so much is known is because of a combination of factors The bodies were buried, fully clothed, in coffins made from split oak logs which were hollowed out, and buried beneath high mounds of turf and soil—the barrows which still today dominate the skylines in many parts of Denmark Gradually the rain water percolated through the mound, and laden with tannin leached out of the oak, penetrated the coffins and thus preserved clothing as well as human skin, hair and sometimes brain tissue

Early discoveries, dating back to the beginning of the nineteenth century, were not particularly well observed and conservation techniques were unable to cope with such delicate material But more recently there have been several notable successes the most fascinating is undoubtedly the discovery in 1929 of a young girl of 20 or so from Egtved, whose midriff-exposing tunic and see-through cord skirt were greeted with shocked incredulity by certain members of the Danish academic world until it was pointed out that several contemporary bronze figures were shown clad in similar skirts

There can be very little doubt, from the surplus energy which must have been expended in constructing the great mounds, and from the richness of the personal equipment buried with the dead, that the coffin burials represent the aristocratic class of bronze age society Glob estimates that the turf required for a single barrow mound would have stripped between two and four acres of pasture of its soil cover When it is remembered that many thousands of these barrows were built in a comparatively short period, something of the devastation of the land can be appreciated

The conspicuous consumption of labour for constructing the mounds and the wealth of the objects of gold and bronze buried within them, metals which incidentally would have had to be imported into Denmark from further south, is a firm reminder of the stability of the native economy, based on the export of local products such as amber, cattle, furs and perhaps slaves, and of the strength of the military hierarchy All this is discussed in great detail in the final chapters, which bring together a wealth of archaeological facts moulded into a cohesive story of a people and their lives, with Glob's customary mixture of insight and intelligent speculation

Professor Glob evidently enjoys his archaeology In this attractively written and beautifully illustrated book he offers us the chance to share his enjoyment The opportunity is irresistible

Necessity for DDT

Chlorinated Insecticides Vol 1 Technology and Application By G T Brooks Pp 249 (CRC Press Cleveland, Ohio, 1974) $30 00

THE number of books on pesticides must by now be big enough to construct a largish pulpit from which to continue the environmental argument over such chemicals in general, and over the chlorinated insecticides, long since cast as the villains of the piece, in particular To those of us involved in agriculture, particularly tropical agriculture, and public health, the banning of these particular chemicals would constitute a disaster, with world food production the victim, the oil crisis may indeed yet cause such a shortage of pesticides as to bring about an unfortunate involuntary demonstration of their beneficial effects to mankind

It is therefore a pity that the various prefaces and introductions to this new series on the chemistry of pesticides seem somewhat apologetic and vague as to the reason why it has been launched "at a time that seems to mark the twilight of the chlorinated insecticides" If men are wise, this twilight will be a long one, because the much heralded "third generation insecticides" will not be a practical economic proposition for many years during which many more million mouths will need filling But during that time we need to re-appraise the potential and refine the deployment of chlorinated pesticides and for this a knowledge of their chemistry will be vital This is adequately and clearly covered in this volume, built up from a very extensive bibliography, included in the account of each group which comprise the DDT group, the diene group, HCH and Toxaphene, is a comprehensive summary of its syntheses and properties and the principles of analysis

The weakest parts of the book are the sections dealing with application, which are mainly a rehash of information already published in such standard texts as A W A Browne and J R Busvine This orthodoxy underrates the vital and increasing importance of correct formulation in application, particularly for the chlorinated insecticides, which can increase their efficiency and reduce their environmental danger, and which must be based on a detailed knowledge of their chemistry

It is difficult to assess the value of the entire book without reference to volume 2, which will deal with biological and environmental effects, but this volume is competent and covers the ground adequately and will be a useful reference work in pesticide laboratories

P T HASKELL

Energy in historical context

Discovery of the Conservation of Energy By Yehuda Elkana Pp x+213 (Hutchinson London, 1974) £4 00

THIS book attempts far more than a straightforward review of the historical development of the concept of energy and of the theory of its conservation Dr Elkana writes in his preface that "the fundamental idea behind [the book] is the essential unity of all aspects of the intellectual enterprise" Thus he discusses not only the internal issues (which in the case of the energy concept are wide-ranging, extending through all branches of science and over a number of centuries) but also the external issues of personality, social climate, metaphysical background and national influence The task he sets himself is a valuable one, I B Cohen, in his foreword, describes him as breaking new ground in trying to resolve the internalist–externalist debate, and certainly if a synthetic view of the historical development of science is ever to be achieved, such a resolution is essential

Despite his somewhat grandiose undertaking, Dr Elkana's book is not a long one, and at the end of it the reader is left feeling a little breathless Many lengthy discussions are curtailed by recourse to the assumption that the reader already has a thorough background knowledge of the extensive literature available, and any aspect of the subject which the author feels has already been adequately covered he omits, giving instead only a very brief critique of secondary sources This seems well justified in a study of this nature, but unfortunately creates some artificial imbalances for the unwary reader Thus, for example, Humphrey Davy is allotted as much space as Faraday or Joule, who traditionally play a greater part

Hermann von Helmholtz is, not surprisingly, the central figure in the book Dr Elkana puts forward the thesis that the very vagueness of the energy concept prior to Helmholtz's work—a vagueness he illustrates elaborately by examples of the confusions centred on words such as 'energy', 'kraft' and 'force'—was an essential element, which, combined with a metaphysical belief in the conservation of some undefined entity, eventually enabled Helmholtz to reach a formal mathematical statement of a general conservation of energy law in the 1840s This then allowed the concept of energy itself to be clarified He emphatically rejects any claims made that Newton, Carnot, Faraday or even Joule had an implicit awareness of the general law before Helmholtz's statement of it He rejects too some of the factors which have traditionally been considered important in the history of the law, such as the search for a perpetual motion machine and the development of the steam engine

The author's tendency to expound his aims and general historical method occasionally becomes distracting, especially since this is not confined to his introduction, but sometimes insinuates itself into discussions of a less general nature Hence interwoven, sometimes irritatingly, with his thesis about the concept of energy is a second, general thesis on how such concepts ought to be studied Occasionally, too, he introduces sweeping historical assumptions which take the reader a little by surprise Nevertheless his approach is very refreshing, and he raises numerous exciting and stimulating questions On every page there are suggestions for further research—indeed the author leaves almost too many avenues unexplored The book should certainly provide an excellent general scheme within which further study of the history of the energy concept can be placed

It is to be regretted that the paper republished as an appendix could not have been incorporated into the main body of the book—or at least printed in type of the same size as that used in the rest of the text

LAURA TILLING

Logic in plant chemistry

Chemotaxonomy of Flowering Plants By R Darnley Gibbs Vol 1 Constituents pp xx+1,680, vol 2 families, pp 681+1,274, vol 3 orders pp 1,275–1,980, vol 4 bibliography, index pp 1,981–2,372 (McGill Queens University Montreal and London, June 1974) $135 per set

Chemistry in Botanical Classification Edited by G Bendz and J Santesson (Proceedings of the 25th Nobel Symposium, August 1973) Pp 320 (Academic Press New York and London, July 1974) $27 50, £13 20

DR GIBBS is the doyen of English-speaking chemotaxonomists, and it is gratifying to receive the four large volumes which he has now produced

After a rather lengthy introduction, the author settles down in volume 1 to 800 pages of chemical plant constituents, arranged alphabetically under headings ranging from acetylenic compounds at one end to waxes at the other, complete with diagrams of structural formulae For the botanist, at least, this provides a very useful dictionary, and it continues to the middle of volume 2, when there is a switch to a second dictionary (of 280 pages), this time of the families of the dicotyledons and monocotyledons, also arranged alphabetically Each family, old or new, is given a paragraph with nomenclatural details and references, and there is an attempt to define the position of the family in the orders in which leading taxonomists during the last 150 years have placed it I found these paragraphs too compressed, often confusing, and of limited value

Dr Gibbs then proceeds to put the chemical and taxonomic information together, to see if it is possible in this way to define more effectively the orders of flowering plants The twelfth edition of Engler's syllabus is used as a basis for the treatment, which extends over the next 800 pages, to the end of volume 3 Orders which are minor or not generally recognised are dealt with briefly, but under each major order the relevant chemical data are given, together with the author's conclusions as to their bearing on the composition and limits of the order Thus, under the Tubiflorae, 26 families are described chemically in 40 pages of text and five tables, and this is summed up in a couple of pages of discussion

The chemical evidence is based partly on published work, and partly on the author's personal tests, which cover a wide range of species The discussions are usually critical and sometimes illuminating, but it is not always easy for the reader to evaluate for himself the great mass of data presented or to follow the author in his reasoning Essentially, he is trying to make sense of the patterns of distribution of chemical compounds among large taxa, and without advanced statistical treatment (and with so many gaps in the information) he cannot get very far But the book will be of value as a storehouse of data and as a record of the author's experience The fourth volume contains the bibliography, the valuable index and some addenda

The book of the Nobel symposium is an excellent piece of work In it, some 30 distinguished specialists present papers on a series of topics, particularly the role of special classes of compounds in the systematics of plant groups of various kinds and sizes, together with critical consideration of the discipline of chemotaxonomy

The papers are all short and to the point, presenting the special data or individual views of the author concerned, and often controversial, and each is followed by a page or so of discussion There are also a general discussion and a summing up The symposium must have been most stimulating to the participants, and this sense of stimulation is communicated in the volume, which is highly recommended

D H VALENTINE

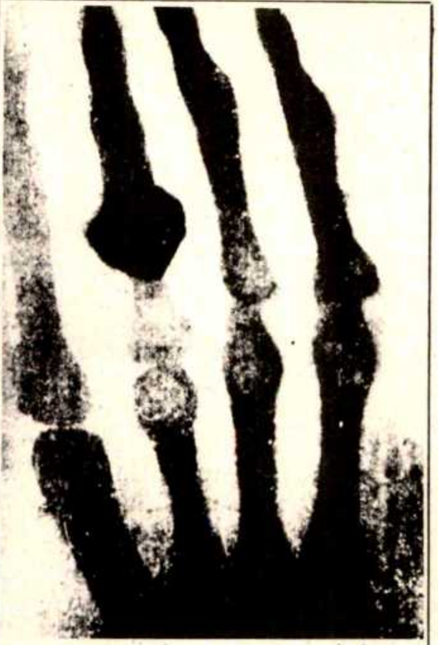
Medical meeting

Medical Research Systems in Europe. Edited by E. P. Woodford. (A joint Wellcome Trust-Ciba Foundation Symposium held in London in March 1973. Ciba Foundation Symposium 21, New Series.) Pp. viii+335. (Elsevier, North-Holland: Amsterdam, London and New York, 1973.) Dfl. 43; $17.20.

WITH all the turmoil in research circles following the British government's acceptance of the Rothschild "customer/contractor" principle of research funding and organisation, this meeting was very timely. Twenty-two speakers from all the major countries in western and eastern Europe except for the Soviet Union circulated in advance papers describing the situation in their countries. This was a wise step, for though the papers are very useful sources of information, a repetition of the various types of support and organisation would have been tedious. The main purpose of the symposium was discussion, and this comprises some 80 pages of fascinating reading, indicating a real exchange of ideas.

Although the meeting was ostensibly about research, it also dealt fully with medical education and administration, and with the ways in which students could be channelled in to research careers. The most obvious differences between the different countries stemmed from their admission policies to medical schools. Most countries, both western and communist, now restrict their student entry to numbers near to those which the teachers think reasonable—the so-called *"numerus clausus"*. Even so there were complaints of overcrowding and difficulties in communication. In Lisbon, where entry is unrestricted, a first-year entry of more than 2,000, with a 50% drop out, was found to be prejudicial to both research and teaching. Several speakers spoke of the danger to the community of the disappointed drop-outs, though this seemed more serious to western than eastern participants. There was acceptance of high numbers of students by C. H. Nachev of Bulgaria, though the proportion receiving maintenance grants there is only 40% of that in Britain.

Nobody was entirely satisfied with the existing situation. Everybody deplored the lack of interest in research by many of the abler students, which seemed more serious than any shortage of funds. In all countries there was some division between work done in universities and separate institutes—where student numbers were high, institutes seemed to play the greatest part. Though the need to cooperate with scientists without clinical qualifications was realised, P. J. Hjort of Norway suggested, not entirely in fun, that, perhaps, "these invaders should be driven back, under the slogan 'medicine for the doctors' ". This sounded somewhat outdated to the ears of those from Britain, where the most eminent of the directors of our most prestigious medical research institutes have been, from a medical viewpoint, laymen. Clinicians and others regretted the difficulties of obtaining the cooperation of patients, though H. Berndt said that in East Germany "patients are well educated and understand the value of clinical research, so they are cooperative".



An early X-ray photograph of "the bones in the fingers of a living human hand. The third finger has a ring upon it." Described by W. C. Rontgen, writing on "a new kind of rays," in *Nature* in 1896. Reproduced in *Patterns in Physics*. By W. Bolton. Pp. vii+448. (McGraw-Hill: Maidenhead, March 1974.) £3.95.

The one thing missing from the symposium was any objective assessment of the success of any particular system of education and research. Experience elsewhere has indicated that there is little correlation between research productivity and available facilities. There was little which suggested that any political system was less—or more—likely to make research particularly productive. R. Saracci of Italy did indeed defend the principle of commissioned research, quoting Peter Medawar's point that painters and musicians in the past produced commissioned masterpieces, so "why shouldn't scientists react similarly?" This book was perhaps of greatest value in providing the background information for a survey of research efficiency, something which has still not been made, even by those who are responsible for the recent drastic changes in research organisation in Britain and elsewhere.

KENNETH MELLANBY

Introduction to superfluids

Superfluidity and Superconductivity. By D. R. and J. Tilley. Pp. viii+262. (Modern University Physics Series.) (Van Nostrand-Reinhold: New York and London, July 1974.) £9.50 cloth; £4.95 paper.

IT has long been recognised that the phenomenon of superfluidity in helium four has much in common with that of superconductivity in metals, each being marked by the macroscopic flow of material particles without dissipation of energy. A major defect of all previous introductory texts has been that although the similarities were in many cases commented upon, a genuinely integrated treatment was not attempted: it is this deficiency which the Drs Tilley have set out to rectify.

They start with brief descriptions of the observed properties of superfluid helium and of superconductors. The macroscopic wave function is then introduced on page 32; and it is around this unifying concept that the rest of the book is built, with chapters discussing the two-fluid model of HeII, electrodynamics and tunnelling in superconductors, vortex states, Josephson effects, Ginzburg–Landau theory, and the possibility of superfluidity in other systems.

The level assumes no more than an undergraduate knowledge of statistical mechanics, electricity and magnetism, and solid state physics, and the mathematical demands on the reader are remarkably modest. It is astonishing how much material the authors have managed to cram into the 248 pages of main text: I suspect that the large concentration of conceptual challenges, while highly stimulating to the beginning Ph.D. student, may prove rather strong meat for the majority of M.Sc. students.

A book of this scope is bound to attract minor quibbles from almost every reader whose particular research interest lies among the topics discussed. It does, however, seem rather a pity that, after a lucid exposition of Landau's famous explanation of superfluidity in terms of the elementary excitation spectrum, the authors fail to mention the one and only physical situation where this approach leads to an accurate numerical value of the critical velocity, namely, the passage of negative ions through pressurised HeII. On the other hand, I very much like the chapter on vortex states, a topic which lends itself particularly well to a unified treatment; and the section which presents a point by point comparison of vortices in superconductors with those in superfluid helium, emphasising the differences as well as the similarities, will be especially useful to novitiates.

A measure of the continuing vigour of research in this field is given by the extent to which the book has been overtaken by important new discoveries during its writing. The most significant of these came with the observation in 1972 of two new and apparently superfluid phases of liquid helium three below 3 mK thus, at a stroke, doubling the number of superfluids known in nature. The authors are to be commended for having managed to slip a page on this topic into the last chapter but, even so, the pace of advance has been such that already the passage strikes one as being principally of historical interest.

Vigorously and concisely written, the volume is lavishly adorned with diagrams and graphs of relevant experimental results, many of which will look comfortably familiar to workers in the field, but a significant number of which have not previously appeared within the pages of any textbook. There is a selection of problems at the end of each chapter except the last. In addition to references at the conclusion of each chapter, there is a select bibliography at the end of the book listing most of the standard texts and laced with descriptive comments, a number of which are amusingly barbed.

Thus in spite of one or two deficiencies, there is so much to be said on the positive side that I, for one, will strongly be recommending my graduate students and, indeed, some of my colleagues to read this book.

P. V. E. McClintock

Analysis of rocks

Laboratory Handbook of Petrographic Techniques. By Charles S. Hutchinson. Pp. xxvii+527. (Wiley: New York and London, 1974.) £10.60.

The title implies a rather unexciting prospect for the reader. Petrography is a vital part of petrology, and few scientists concerned with the genesis of rock complexes can afford to neglect the textures and mineral associations within a rock sample. Even so, it is the petrological analogue of anatomy in medicine, and has recently been in danger of neglect during the race by earth scientists to embrace geophysics and geochemistry as providing the main information for understanding the evolution of the Earth. Petrographic analysis is not essential for all geological studies, but there are few in which it does not demand an important role.

Some petrographic techniques have remained essentially unchanged throughout this century, such as polarised-light microscopy, mineral separation, and determination of specific gravity and refractive index. There have, however, been advances in method and this book presents an up-to-date survey of the whole field. The real advance made here, compared with earlier books on the subject, is the inclusion of sections on X-ray diffraction, differential thermal analysis and thermoluminescence measurements, and the expansion of the concept of petrography by including geochemical analysis by X-ray fluorescence, atomic absorption, and other techniques for components that cannot be analysed by physical methods. An excellent chapter on "Display of Data" deals rigorously with norm calculations, variation diagrams for rocks and minerals, and the statistical analysis of data.

The book is designed as a laboratory manual, and step-by-step instructions are given for all the techniques, ranging from photomicrography to the determination of feldspar structural state, matrix corrections in XRF spectrometry, or the calculation of mineral formulae. The subjects are well illustrated, and the book makes interesting reading. The author has, in fact, been remarkably sucessful in bringing new life to an old subject, probably because he conveys the impression that each technique has been subjected to his critical usage, and because he makes lively quantitative recommendations that are difficult to fault.

There is some overlap with *Physical Methods in Determinative Mineralogy* (edit. by J. Zussman) and *Methods in Geochemistry* (edit. by A. A. Smales and L. R. Wager), although surprisingly he does not refer to the latter. Nevertheless, the emphasis of this book is different and there is no real equivalent for a research student or advanced-level undergraduate specialising in petrology. It will save supervisors and tutors many hours of routine instruction, and may extend the life expectancy of much laboratory equipment.

My major criticism of this otherwise excellent book is the inclusion of 68 pages on X-ray fluorescence spectrometry which, like electron probe analysis, is dealt with in specialist texts. The author omitted electron probe techniques partly for that reason, whereas I would view them as vital to petrographic analysis. In contrast, the section on reflected-light microscopy is absurdly brief (about 6 pages) despite its vital role in petrography and the difficulties encountered by students.

Other notable omissions, in the context of the author's aims, are single-crystal X-ray diffraction (for example anorthite structural state), petrofabric analysis, factor analysis, electron microscopy and Mössbauer spectroscopy. Some attention to problems of zoned crystals (refractive index section) and modal analysis (for example use of reflected light), and to mineral examples (eight pages for garnets versus one third of a page for clinopyroxeies, under X-ray diffraction) are also needed, in any revised edition.

G. M. Brown

Please take note

Effective Technical Writing and Speaking. By Barry T. Turner. Pp. xi+206. (Business Books: London, 1974.) £4.75.

The Professor of Industrial Management at the University of Newcastle has aimed his book at engineers and scientists in industry; there are chapters on how to write specifications, sales literature, contracts, patents and handbooks. But the sections on writing articles and reports, on organising meetings, on giving talks and how best to illustrate them, will interest academics as well.

Professor Turner's advice on all these topics is sensible and helpful. Some of it might even seem obvious, were it not for the incomprehensible handbooks, the turgid and jargon-filled articles, and the ill-prepared lectures with illegible slides, that we are all unhappily familiar with.

We are urged to avoid technical jargon in our writing; to use short sentences and short Anglo-Saxon words; and to approximate to conversational speech. It helps, we are told, to "write in haste, polish at leisure". A few false grammatical idols are toppled: " . . . grammar is common speech formulated—usage is the only test" (Somerset Maugham). The Professor disapproves of foreign phrases, which is unfortunate because "*le mot juste*" appears on page 2—professorial licence perhaps.

There are a few misprints in the book, some of which are amusing: ("The medium is the massage"; MacLuhan). More serious is an error in a table on SI units, in which kilo, mega and giga imply factors of 10^6, 10^9, and 10^{12}, respectively. Recommended reference books do not include a dictionary of quotations, which is perhaps why George Eliot is misquoted. And in a book devoted to better writing the use of "disinterested" to mean "uninterested" is regrettable.

In general, however, Professor Turner's book makes useful reading for anyone speaking or writing on technical subjects. It would be a profitable addition to any library.

John Walker

Revolution in physics

The History of Quantum Theory. By Friedrich Hund. Translated by Gordon Reece. Pp. 260. (Harrap: London, March 1974.) £6.10.

THE main part of this book is a blow-by-blow account of the development of quantum theory in about the first 28 years of the present century, from Planck's introduction of light quanta through the 'old quantum theory' up to the formulation of quantum mechanics and wave mechanics culminating in Dirac's relativistic equation for the electron. The whole of existing basic quantum theory may be said to have been produced in about the three years 1925-8. The school of Max Born in Göttingen, including Heisenberg, Jordan and Pauli played a leading part at that time and Professor Hund was himself a prominent member. So here we have a first-hand account of all that was achieved in those heroic times of phenomenal activity, as well as the story of what had led up to this. It would be almost impossible for anyone ever again to provide such a survey, and the history of science will owe a great deal to Professor Hund for giving it.

The important thing is that this account has been written. It would, however, have been interesting to know more about the history of the history. The brief foreword is undated and there are no bibliographical particulars whatever of the original German publication save for its title: *Geschichte der Quantentheorie*. There are a few references to relevant items in the literature after about 1930 up to the present, but the text could have been written any time in that interval.

Within its set design, the history is comprehensive and exceedingly well digested. It is clear and it is also very terse. Its value as a historical document is all the greater for these reasons. But they almost inevitably eliminate the excitement and romance. Before the first World War there were tremendously revolutionary ideas about atoms, photons, space and time. After that there was an interlude when all kinds of things about atomic spectra—transition rules, line intensities and the like—were got with extreme ingenuity by using a wierd combination of classical and quantum physics with astonishing elaboration and success but with no new basic idea. Then suddenly quantum mechanics in its several varieties came into bloom and fruition; its doing so seemed curiously inevitable though the forms were wholly unexpected. The facts are all here on permanent record; however, there has never been a time like that in the history of physical thought and one doubts whether the reader in a different epoch will be able to re-create from the bare facts the stimulus of living through it.

Also I am convinced that no historian has yet explained how there came to be such a rush of discovery by so many scientists in so many places all at the same time. All previous fundamental advances seem to have been largely the work of one outstanding individual at a time—one may think of Newton, Maxwell, Rutherford, Einstein, Bohr as examples—but in 1925-8 fundamental contributions came from maybe 20 individuals of comparable stature (no doubt some more comparable than others), several of them only half the age of the others, working in some 10 or so different institutions. Hund does indeed indicate these aspects in a brief note on "Centres of Research" at the end of his book; it contains a most interesting diagram on page 251 showing the times when a number of the leading workers made their contributions, but this is presented without explanation or comment. Possibly someone else, working from all this material so ably marshalled by Professor Hund, will some day succeed in revealing the underlying historical causes of the phenomenon.

The book is expensive for its number of pages, but perhaps not for the concentration of information they convey. Gordon Reece's translation is outstanding. W. H. MCREA

Some useful ammunition for worried ornithologists

The Seabirds of Britain and Ireland. By Stanley Cramp, W. R. P. Bourne and David Saunders. Pp. 287. (Collins: London, 1974.) £3.50.

HERE is a classic for the last-page-first reader since the final appendix clearly illustrates both the origins and the uses of this book. In this appendix figures are given which demonstrate the fate of puffins at various breeding colonies in Britain and Ireland during the last 100 years or so. The immense colonies on so many of Britain's islands at the turn of the century are all seriously depleted or even deserted; the 20,000 birds on Puffin Island, County Kerry in 1965 had declined to 3,700 five years later. Not that all is gloom with Britain's seabirds. As this book shows, gannet, kittiwakes and fulmars have all had population expansions, even explosions, in recent decades. Not only are the causes of these fluctuations unclear but all too often their extent is little more than guesswork. In order to build a sound basis for assessing future fluctuations, the Seabird Group, founded in 1965, took upon itself the onerous task of making as full as possible a census of the breeding seabirds of Great Britain and Ireland. After a pilot study in the year of the Torrey Canyon disaster, the full survey was carried out in 1969–70. "Operation Seafarer" as it was known, yielded several volumes of detailed results. The essence of these forms the basis of this book.

Twenty-four regularly breeding species are considered under the title of seabirds although such thoroughly maritime birds as the eider duck and the oyster catcher are arbitrarily excluded. The bulk of the text is composed of a section devoted to each species and covering their identification, breeding, world distribution and past and present status in the areas surveyed, together with a note on the census method used for each species except, inexplicably, any of the gulls. These sections are sound and well referenced. Only the identification details, even in conjunction with the rather mean drawings and photographs, leave something to be desired; but they are probably superfluous anyway.

The rest of the book consists of three chapters on seabirds—dealing with their biology, the threats to their existence and their present numbers and changing fortunes. These make informative, if not always elegant, reading. At the back of the book are placed a series of maps and tables. The former, one per species, show the locality, number and size of the breeding colonies discovered in Operation Seafarer. Crispin Fisher is to be congratulated on presenting in them a large deal of information in a masterly way. The tables are an inconsistent set, sometimes supplementing the data in the maps, sometimes duplicating it.

Much of the information in the book is unique, all will be valuable. It is perhaps a pity that room could not be found for a more general chapter on bird population changes covering at least Europe and including inland as well as seabirds. It would also have been fascinating to know more about the organisation and logistics of the survey. We are told something about the census methods, which ranged from the smelling out of storm petrels' nesting sites to aerial photography of gannet colonies. But exactly how the hundreds of intrepid observers worked we never find out.

Despite some deficiencies this book should appeal to all who have more than just a passing interest in birds. As the sides line up for forthcoming battles over North Sea oil landing sites and other coastal attacks, it will be a source of much useful ammunition.

PETER NEWMARK

Culture and cognition

The Cultural Context of Learning and Thinking: An Exploration in Experimental Anthropology. By M. Cole, J. Gay, J. A. Glick and D. W. Sharp. Pp. xx+304. (Tavistock: London, June 1974.) £1.50.

THIS book is an important attack on the problem of the relation between culture and cognition. Too often in the past psychologists have travelled the world with a kit of psychometric tests, showing that on most tests "primitive" people obtain very low scores. They have concluded either that these people are innately less intelligent than those in western society, or that aspects of their environment have hindered their intellectual development. In both cases, what is posited is an intellectual deficit, a failure to develop the most powerful cognitive structures. Recently, however, a number of psychologists have argued that cognitive skills of the same order develop in all human groups; what differs between groups is the contexts in which these skills are applied. In order to assess the skills of another culture it is necessary to understand the tasks it imposes on its members. This book describes an interdisciplinary study by anthropologists, linguists and psychologists of cognitive skills in one African society.

The authors started with an educational problem; the Kpelle children in Liberia experience great difficulty with western type mathematics. Yet in their daily lives the Kpelle use quantitative operations and argue skilfully and with logic. In order to resolve this paradox the authors developed a series of experiments that would throw light on the Kpelle concept system and their methods of learning and problem solving. They first attempted to describe the Kpelle classificatory categories, and then used them in their investigations of learning, remembering, and concept formation with groups of non-literate children and adults, and primary and secondary school children.

A brief account of one investigation will give the flavour of the work. Kpelle schoolchildren seem to learn by rote, without grasping the principle involved. The authors confirmed this observation experimentally; in classical learning tests the initial recall of the Kpelle was poor, and increases with practice were negligible. These deficits seemed to be related to their failure to impose any order or structure on the material to be learned. Performance was no better when objects were substituted for words. The authors sought ways of evoking efficient performance. Attempts to improve motivation by a variety of rewards were unsuccessful: however, when the task was structured, either by indicating the categories involved, or by embedding the words in a story, recall dramatically improved. Only the high school students could impose structure on the learning material themselves.

From these and many other experiments, and from a study of Kpelle society, the authors conclude that the non-educated Kpelle is capable of concept-based learning, inference and logical thought, but that the situations in which he applies these skills are different and more restricted than those of his high school age mate. The findings raise many questions—for example, what learning experiences are crucial in order to develop transferability of skills—and this important book should stimulate more work by educationalists and psychologists.

BARBARA TIZARD

A theoretical view of life

Molecules and Life: An Introduction to Molecular Biology. By Mikhail V. Vol'kenshtein. Translated from the Russian by Serge N. Timasheff. Pp. xiii +513. (Plenum: New York and London, 1974.) $9.55.

MOLECULAR biologists at work may be compared to industrial archaeologists: pieces of the machinery are carefully retrieved, dusted and measured and parts of the mechanism are put together in the test tube and made to work. This sort of activity has been in full swing for fifteen years or more and has led to an impressive, though far from complete, understanding of what makes living things tick. In the background of this activity have hovered physicists and theoreticians who have been crucial in inventing and applying new methods of gathering information and, more peripherally, who have attempted to explain the properties of interesting biological components in terms of fundamental physical theory. The latter have often impressed and sometimes enlightened the less sophisticated biochemist but I would guess that they have rarely blazed pathways for molecular biology.

Academician Volkenshtein has made important contributions to our understanding of polymers and several other fields and has in recent years turned his interest to biology. One of the fruits of the latter interest is this volume—first published in the Soviet Union in 1965 and now available in revised form in English in a paperback edition. As the author says in his preface the book deals principally with the structure and function of nucleic acids and proteins but, throughout, the quest of the theoretician for regularities and generalisations is apparent. This is first manifest in the first chapter which is devoted to grand concepts such as statistical mechanics, irreversible thermodynamics and information theory. These notions and their application to biology are explained with admirable clarity but one is left with the feeling that they are not of immediate relevance to the working molecular biologist in that they tend to disappear into abstraction. To take one example, an argument is presented in refutation of the hypothesis of the inheritance of acquired traits in terms of the proposition that no flow of information from somatic to sex cells has been detected. I am sure he is right but an experimental fact or so might have been more convincing. In similar vein, the text which is otherwise descriptive breaks into mathematical derivation and matcrices and their Eigen values loom up from the pages. These may deter the biologist and it would be better to have a degree in physics or chemistry with some mathematical experience before embarking seriously on this book. But this is not meant as a criticism and more experienced workers will find much to interest and enlighten them in this book for topics not covered in similar works are given space; simple treatments of helix coil transition theory are hard to find and the sections on the genetic code and on polymer conformation are especially interesting.

Nevertheless, the graduate student entering the field for the first time and to whom I hesitate to recommend this book should beware of several errors and misleading passages. One of the more serious occurs in the chapter on protein structure. There, the idea is presented that the success that Perutz achieved in the X-ray crystallography of haemoglobin by using heavy atom derivatives had something to do with making the diffraction spots sharper and revealing the location of the hydrogen atoms, rather than being the crucial method of overcoming the phase problem that it was. In the chapter devoted to a cytological description of the living cell, no prominence is given to the distinction between prokaryotes and eurkaryotes and the significance of the single sentence that says that bacteria do not have a visible nucleus is unclear.

Again, as is to be expected in a book first published in 1965, much recent work is not mentioned and hence there is little on ribosome structure, protein synthesis or the structure of tRNA, to mention just a few topics. In fact there are few references dated later than 1965 so to read this book in isolation would give a somewhat antiquated picture of the state of molecular biology. One would question the wisdom of issuing a paperback edition of a book first published nine years previously were it not for the distinctive flavour of the approach and the light it may throw on thinking on molecular biology in the Soviet Union.

E. G. RICHARDS

nature

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Doing something for the centre

THERE is something distinctly unnerving about writing about the British General Election before it happens yet being published only after it is all over. The one thing that the last two elections have clearly indicated is that it would be the height of folly to assume even two days before the event that any particular party had won. The most recent opinion polls unanimously point to a Labour victory with 42% of the vote compared with the Conservatives' 35% and the Liberals' 19% and if this pattern persisted until polling day a Labour government would be returned with a majority of at least fifty over all other parties. Certainly the pattern has shown a stability over the past few weeks which reflects the inability of any political party to make a major impact on the uncommitted, but there is a feeling that there is still all to fight for.

The issues before the electorate are more serious than they have been at any time in the recent past, and the party positions are probably as clear-cut and distinguishable as they have ever been. And yet it is this very clearness of division which has made the choice for anyone who gives some thought to his decision an extremely difficult one to make this time. For separation on the issues, particularly on how to handle inflation and on how to fortify British industry, inevitably means bringing out that perennial skeleton in the cupboard—class warfare. Both Labour and Conservatives have, of course, their mechanisms for moderation; the Labour party's social contract with the unions looking at present deservedly more like an election-winner than the Conservative pledge to create a government of national unity. And yet the social contract, a sort of Swedish arrangement with the unions to work for more equality in exchange for moderation in wage claims is not universally enthused over by the unions, particularly those which represent the more-than-equal at present. And if anything there is more socially divisive electioneering from Labour than there was in February, and there have even been threats of industrial chaos this winter if Labour does not win.

Not that the Conservative party has actually been proposing policies which seem to be aimed at picking up the floating voter who might be disturbed by the threatening noises from the left, but fears that old-style Conservatives would get the country back into confrontation politics between government and unions. There is as yet no sign that old-style Toryism is being replaced by something more of the centre.

And yet it is pursuit of the centre which both of the present major political parties ought to be engaged in at a time of deep crisis if they seek electoral success; surely the McGovern experience ought to have taught moderates the world over that lesson. In any country with a less perverse electoral system it would be ridiculous that the support of 42% of the populace should give an unfettered mandate to a party, and it is high time that if the two major parties do not move to the middle ground, life should be made easier for the Liberals—who have been occupying it without success for so long.

The dilemma for the voter contemplating the Liberals has always been the taunt that a vote for them was a vote wasted, and yet even against this they have made steady progress until this election. It is unlikely that they will make much more headway now for rather obvious reasons. Their policies are widely regarded as attractive, sensible and likely to unite the country but their abilities are bound to be suspect whilst they have a mere handful in parliament but an army of untried hopefuls outside. There are many able politicians of the centre at present in the two main parties who, one suspects, would happily defect to the Liberals given some job prospects. The party finds itself in the bind of having amassed the support of a fifth of the electorate, the potential for more and yet, without being given some sort of break, it probably will be incapable of going above the critical size (at about 28% support the party would probably have a hundred or so members in parliament).

The case for letting some fresh air in to the British parliament is strong and it is only just that a party of the centre with a broad support should provide much of this fresh air and help towards realigning British party politics in a more healthy way. But this is unlikely to occur in the near future unless electoral reform is taken seriously by one of the two main parties. The question of which is the best of many systems of representatives tried elsewhere should not be allowed to bog down the central issue of the need for reform. And if in the next few years neither party comes to terms with electoral reform, it will be incumbent on the more thoughtful and moderate to lobby intelligently for social justice at the very place which claims to dispense social justice.

A hundred years ago

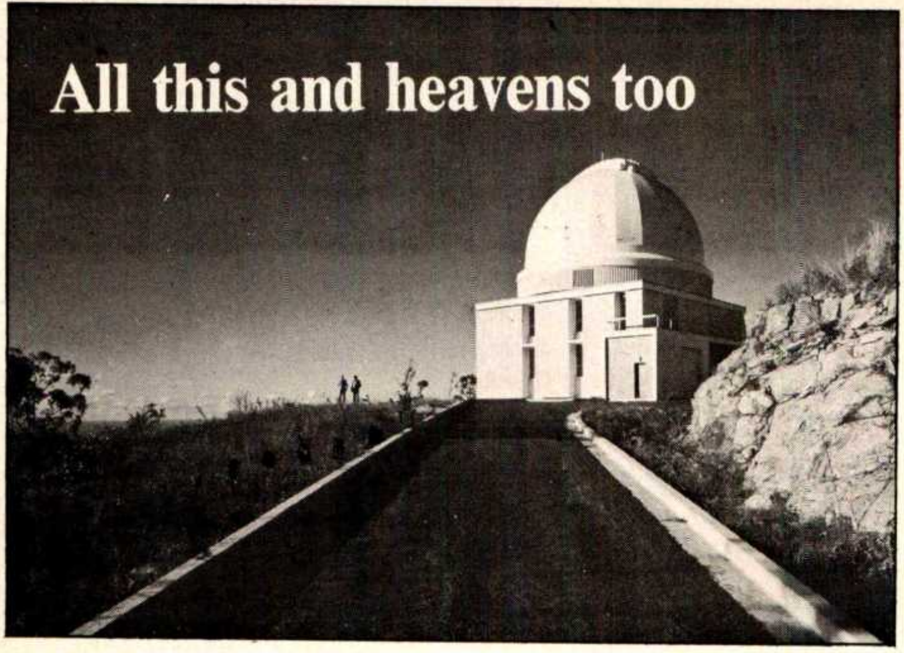
An Anagram

THE practice of enclosing discoveries in sealed packets and sending them to Academies, seems so inferior to the old one of Huyghens, that the following is sent you for publication in the old conservated form :—

$A^8C^3DE^{12}F^4GH^6I^6L^3M^3N^5O^6P$
$R^4S^5T^{14}U^6V^2WXY^2$ WEST

From *Nature*, **10**, 480, October 15, 1874.

All this and heavens too



On October 16 the Anglo-Australian 150-inch telescope is being inaugurated at Siding Spring in New South Wales. Together with the recent announcement of plans for an infrared flux collector in Hawaii, the imminent retirement of the Director of the Royal Greenwich Observatory and of the Astronomer Royal for Scotland, and with the concept of a Northern Hemisphere Observatory very much alive we are clearly at the beginning of a new era in British astronomy. John Gribbin has been talking to Dr Alan Hunter and Professor F. G. Smith, of the RGO, and Dr V. C. Reddish, of the Royal Observatory Edinburgh, about future prospects in the light of these developments. Picture: dome of UK 48-inch Schmidt telescope at Siding Spring.

IN terms of optical astronomy, Britain has been behind the United States since the beginning of this century. The 60-inch telescope completed some 70 years ago and other telescopes on good sites within the home territory of the United States made this inevitable; and modern astrophysics needs big telescopes. Only 130 years ago, of course, the biggest telescope in the world was in Ireland. But that was no consolation to the bright youngsters in Britain who were interested in astronomy in the 1920s and later. The natural reaction of such people was to move into theoretical work, where thanks to Eddington and others Britain became as pre-eminent as the United States was in observational work.

And that, of course, led to a still more lopsided growth of the subject in Britain. The next generation followed where these pioneers had led and achieved fame, and what could have been a very unhealthy situation was saved only by the advent of radioastronomy.

In recent years there have sometimes been bitter attacks on the administrators of British science for failing to prevent Britain slipping behind in terms of optical telescopes. But much of this criticism, made only now with the benefit of hindsight, seems a little harsh when we recall the conditions which prevailed, say, 30 years ago. Dr Alan Hunter, who retires as Director of the Royal Greenwich Observatory (RGO) next year recalls that in 1947 it was still something of an adventure to travel to South America to view a solar eclipse. In those days, the aircraft still flew south on the first leg of the journey, in order to cross the Atlantic at its narrowest point. Yet the decision to build the Isaac Newton Telescope (INT) in Sussex was taken in 1946; could the planners really have been expected then to appreciate how jet travel would revolutionise astronomy, opening up the prospect of British telescopes being run effectively even when built in Australia or Hawaii?

The experience now being gained with the Anglo-Australian Telescope (AAT) and the UK Schmidt camera on the same site illustrates how rapidly things have changed over the past decade. Dr V. C. Reddish, of the Royal Observatory Edinburgh (ROE) has been closely associated with both projects, and is at present on the management committee of the AAT. The Schmidt camera has now been operating for a year, and he says that the first papers reporting observations made with the instrument will be published soon. The importance of this camera, and the reason for siting it with the AAT, is that it produces a wide angle view of the sky, covering twelve times the Moon's diameter on one plate. Essentially, such a camera is used for survey work while the more powerful but narrower angle optics of the 150-inch will be used to investigate interesting sources revealed by the survey. The Schmidt plates are also of great interest to radioastronomers who wish to identify the sources they have found.

In the northern hemisphere, the Palomar Sky Survey (obtained with a 48-inch Schmidt camera) has provided the basic sky atlas for astronomers over twenty years or so. But there has never been a comparable survey of the southern sky.

This is being rectified by the UK Schmidt and by a similar instrument at the European Southern Observatory in Chile (ESO). Each camera is taking a set of plates covering the whole southern sky, but at two different wavelengths. Together, these plates make up an atlas which enables astronomers to pick out many interesting objects—such as possible QSOs—from their relative brightness at two frequencies. Reddish says that the operation of the joint survey with ESO has been "co-operative, pleasant and efficient". ESO plate copying facilities in Geneva are being used for both ESO and UK Schmidt plates, and this specific area of collaboration typifies the way astronomy has developed as an international science.

The UK Schmidt has given British astronomers experience of both international cooperation and of the building and running of a really big telescope. Of 800 plates produced in the first year of operation, more than 100 have been accepted for the southern sky survey. This reflects a wish to maintain high standards, since plates once accepted for the survey will be used by many people for many years, together with a reluctance to seek perfection, which would make the task impossibly long.

Even so, thanks to the advances made since the Palomar Sky Survey was carried out in the north, the new Schmidt is producing plates which are a distinct improvement in quality over any previous survey. Thanks partly to the use of a new photographic emulsion (known as IIIaJ) and a new technique for treating plates before exposure by soaking them in oxygen-free environment (*Nature*, **249**, 22; 1974), the plates record all celestial objects down to magnitude 23 with an exposure of 40 minutes. This means that twice as many radio sources can be identified as on comparable Palomar Sky Survey plates, and that radioastronomers can even tell what type of galaxy their sources are associated with at magnitudes which would not even be recorded on Palomar plates.

There is no direct association between the building of the Schmidt and of the AAT, but inevitably some expertise must rub off from one committee to another, since some people, like Reddish, have been involved in both projects. Reddish points out that although the AAT will be the third biggest telescope in the world it has "superb" optics and that, together with the improvement of instrumentation over the past 30 years, means that it could well be even more powerful in practice than the 200-inch, just as the 48-inch UK Schmidt is more powerful than its older counterpart at Palomar. Already there is great demand for time on the AAT, and even some very good projects will inevitably fail to receive an allocation.

The attitude of the Science Research Council (SRC) certainly seems more realistic than they have sometimes been given credit for. Accepting that a capital investment of £1 million must be used efficiently, they are prepared to fly people out to Australia to work with the new telescope two or three times a year. And there may well be more large capital investment in the offing, again at sites far from Britain.

Already the decision has been made to go ahead with construction of a 3.8-m infrared flux collector in Hawaii (*Nature*, **250**, 617; 1974), and a decision will be taken in the new year on whether to build a UK Northern Hemisphere Observatory (NHO). This idea has been in the wind for several years, but should now receive the go ahead. France, Germany and Italy are all planning their own large telescopes, and although this activity might seem to run counter to the spirit of international cooperation in astronomy that is not really the case.

According to Dr Reddish, it is not efficient to share construction of telescopes and observatories too widely—the technology of interfaces are difficult enough to cope with without the worry of international management interfaces. But it does make sense to share sites, services and overheads. Through that kind of sharing joint use of the instruments will inevitably follow.

All that, together with the infrared telescope decision, must make Hawaii one of the favourite contenders for the site of the NHO. That site is really a little too high, since it becomes difficult to breathe on top of the Hawaiian mountains. But otherwise it seems ideal, with a US observatory already there and obvious possibilities of sharing the running expenses. With such an observatory—in Hawaii or elsewhere—Britain could well, in fact, lead the world in observational astronomy for the rest of this century.

That would certainly be a remarkable development. But it reflects the growing awareness that astrophysics is rapidly becoming the big science of the present day. Both Reddish and Professor F. G. Smith, who has just left the University of Manchester to go to the RGO as Deputy Director, and eventually to succeed Dr Hunter as Director, commented on the high standard of people entering astronomy today. There is no evidence of a drift away from the sciences here; in fact, the very best students now are of a higher calibre than ever before.

This is understandable. It is now clear that the next great advances to be made in physical science are likely to centre on problems of high energy and the study of gravitation. And these problems cannot be tackled in the laboratory. Nuclear astrophysics, cosmic ray studies, and information about energetic sources obtained at all parts of the spectrum are the 'laboratory' sciences of the immediate future. It is no wonder, then, that the first class physical scientists today are attracted primarily to astronomy.

Indeed, Smith's move from Manchester to the RGO is one symptom of how astronomy's face is changing. Smith emphasises that he is an observer, not a theorist, but that there are no barriers between different astronomical observers any more. With modern instrumentation, whatever kind of 'electromagnetic radiation bucket' is used to collect signals, the job of the observer is really one of analysing electric impulses. So it is misleading to talk about 'radio', 'optical' or 'infrared' astronomers; as Smith says, "we are all just 'astronomers' now".

Even so, there must be a good reason for Smith to move to the RGO at the present time, and it seems that the lure of the NHO has played a large part in this. The RGO has the job of creating and operating the NHO (assuming it receives the go ahead), and could well become the most important astronomical centre not just in Europe but in the world—indeed, the way science is progressing at present, one might well envisage the RGO becoming the key scientific centre in the world. It is small wonder that Smith should be attracted by the challenging job of acting as Director of the RGO for the exciting years ahead.

Both the RGO and the ROE have university links, the ROE a long established relationship with the University of Edinburgh and the RGO a more recently established but important link with the University of Sussex. The RGO also provides summer schools in astronomy for university students from all over Britain, and these are so popular that only one applicant in five can be accommodated. In Edinburgh, when a job on the UK Schmidt was advertised recently there were ten applicants, nine of whom had first class degrees and were short listed. This is unprecedented in Reddish's experience; astrophysics is now getting the pick of the research students in the same way that nuclear physics did 50 years ago. Looking at the progress made in nuclear physics since the 1920s, that simple statistic, as much as anything else, points to an exciting time for British astronomy in the next 25 years.

The Lesser Magellanic Cloud: photographed through the UK Schmidt telescope.

Courtesy of the Science Research Council.

international news

“*Nature* published two replies (June 14, July 5) to the letter from Dr Gerschenfeld and his colleagues (March 29) dealing with the dramatic conditions of the Chilean academic community. These replies support the military junta, minimise the abrogation of civil liberties and democratic government in Chile, and suggest that Dr Gerschenfeld and his colleagues were not properly informed. Coming from supposedly well-informed Chileans, they leave the impression that the situation there has indeed been over-dramatised. In fact both replies are studded with misleading and tendentious statements we feel obliged to correct. Most of us worked and lived in Chile under the government of Dr Salvador Allende and during the military coup of September 11. We feel qualified to write about conditions in the Chilean academic community at least as much as Admiral Swett, who had no relation with academic life before his appointment as rector of the Catholic University, or Professor Eyzaguirre who has lived for the past 17 years in the United States.

Admiral Swett admits in his letter that he was nominated rector by the military junta, which is extralegal. To minimise this abuse of power he adds that he was confirmed by the head of the Catholic Church. However, the Catholic bishops of Chile in a collective declaration of April 1974 entitled "The reconciliation in Chile" have publicly stated their concern for ". . . arbitrary arrests or imprisonment by ideological motives . . ." and declared that Chileans are "living in a climate of insecurity and fear". We believe that the head of the church had no alternative to accepting the nomination of Admiral Swett. Professor Eyzaguirre repeated this and other remarks, but in a very different style. He spent half of his letter discrediting the Allende government and justifying the military coup, expounding the official statements of the military junta. This is a general polemic, and irrelevant to the specific charges presented in the Dr Gerschenfeld's letter and under International News in *Nature*, May 3. Few factual statements were challenged in the two "replies from Chile". We wish to comment on those few challenges since they definitely distort the reality.

"Students at some universities had to reregister. Students who did not qualify as such in the traditional sense (. . .) were dismissed. This weeded out the purely political 'students,' . . .". In fact, all students at five out of six universities had to reregister. 20,000 students were dismissed. Since more than a thousand lacked a few credits or a final examination to obtain their degrees and had entered the university two to four years before Allende was elected president, it is difficult to see them as purely political "students".

In reply to recent letters in Nature from supporters of the Chilean junta, twenty-six Chilean scientists have signed this joint statement on the condition of academics in the country. Their names have been supplied to the Editor.

"The Faculty went through the same screening procedure. Those who did not qualify for lack of degrees or for being exclusively political activists were dismissed," wrote Professor Eyzaguirre. More than 60% of the Faculty of the Technical University, almost the whole Faculty of the schools of Anthropology, Psychology, Sociology and Economics at the University of Chile, Concepcion and others, and most of the members of the prestigious Department of Public Health of the School of Medicine at the University of Chile would then be exclusively political activists. Admiral Swett said "They were dismissed because they simply abandoned their jobs. . .". Many of us were indeed dismissed for abandonment of our jobs but it should be known that we were absent because of imprisonment.

"Very few fell either immediately after or during the coup because, allegedly, they were caught either shooting at soldiers, carrying arms or actively participating in the organisation of revolutionary activities." We and almost every colleague know that professors Mario Ramirez, Jorge Pena and Enrique Paris neither shot at soldiers nor carried arms; we must infer that they were executed because of "organisation of revolutionary activities"; a very broad charge since the government of Allende was categorised as revolutionary.

"There are charges against some of these people and if they return they will face legal prosecution. But prosecution is one thing and persecution another." It is general knowledge that legal procedures have been suspended in this instance. Dr Edgardo Enriquez, minister of education and former rector of the University of Concepcion; Mr Enrique Kirberg, rector of the Technical University; Mr Galo Gomez, president of CONICYT; Mr Raul Iriarte, secretary of CONICYT, and many other less known members of the academic community have been imprisoned from September 1973 with no formal charges and no trial date.

"Some academicians have had trouble with the police because of denunciation by neighbours or colleagues. These people have been investigated . . . " Investigation consisted in arrest and interrogation usually with physical mistreatment. Many of us experienced this and it was clear to us that this was purely political persecution.

"The appointments . . . (of retired senior armed forces officers as rectors) . . . would not be too far from practices that are not unknown in the United States." The appropriate parallel to the Chilean situation would be the appointment of military presidents in every American university, by the Pentagon.

"Chaotic finances which prevented normal university activities and the payment of adequate personnel salaries" (during Allende's government). There were indeed some financial problems for adequate scientific research but during this time the universities received their largest share in the history of the national budget. This policy should be compared with that of the junta which ordered every university to finance itself with no funds from the government by January 1976 while the share of the armed forces has been increased from under 10 to 40% of the national budget.

With regard to Chilean scientists remaining in Chile, we agree that they are not fascist (nothing close to that is said in Dr Gerschenfeld's letter) but people sincerely devoted in pursuing their research under very difficult circumstances. We believe, however, that the letters of Admiral Swett and Professor Eyzaguirre do not help them and are in fact unfair since these colleagues are not in a position to disagree. Chilean scientists remaining in Chile as well as those who will return are better helped by encouraging the return of at least academic freedom.”

● THE COURT of inquiry into the explosion which killed 28 people and caused £70m damage at the Nypro chemical works in Flixborough, Lincolnshire, has been told that a temporary by-pass system in the cyclohexane unit was not tested at an appropriate pressure. According to counsel for the company (owned jointly by Dutch State Mines and the National Coal Board) it is now clear that the by-pass system was installed without an adequate design study, and that if such a study had been carried out, a different system would have been used. Most of the technical evidence has yet to be presented, and a recess is expected to allow for a consensus of expert specialist opinion. Reaching the consensus, however, will be difficult because of the quantity and complexity of the technical information likely to be available.

But there is no doubt that the findings of the inquiry will have profound implications for the chemical industry in Britain and elsewhere because of the fundamental safety issues which have been raised. The complexity of chemical plants and the huge reserves of stored energy they contain seem to have completely outrun the rather Victorian arrangements of the Factories Inspectorate—the 1974 Health and Safety at Work Act notwithstanding. The safety officer at the Flixborough site told the court that nothing on the scale of the explosion had ever been conceived or considered and that the rule book governing the safety of chemical plants needed rewriting in the light of events there.

The Health and Safety Commission now being set up is bound to look carefully at the results of the findings of the Flixborough Inquiry. As an independent commission it will bring together the existing government inspectorates covering nuclear installations, explosives, alkali works, mines, quarries and factories. The commission was proposed before the events at Flixborough but it comes at a time when a lead to industry is desperately needed. Already some large petrochemical companies have approached the Atomic Energy Authority Safety Commission for consultation on safety problems and there is a growing feeling, not least amongst the companies themselves, that the chemical industry should be governed by regulations at least as stringent as those of the Nuclear Installations Inspectorate.

Round Britain

● IN SPITE of recent inflammatory news reports, vaccination is still the best means of ensuring overall community protection from infectious diseases. This was the outcome of a report on vaccination published by the Office of Health Economics (OHE) last week.

While diseases such as polio, diphtheria, tetanus and measles still present significant health hazards, OHE maintains that in some areas of the UK, only two-thirds or less of all children are adequately immunised. Thus to maintain public trust in vaccines available from the National Health Service, withdrawal of controversial vaccines such as whooping cough (pertussis) may become necessary in the near future. It has been claimed that pertussis vaccine has caused brain damage in a small number of young infants, although there are no published figures to substantiate such an accusation. The OHE, however, recognises that an element of risk is associated with vaccination, but takes the view that provided the benefits far outweigh the risk then it is acceptable.

The study suggests that only a very small proportion (probably a few hundred) of the 50,000 to 60,000 severely mentally handicapped children in Britain owe their condition to immunisation by pertussis vaccine. But this small proportion has become of increased relative significance because of the fall in risks from other factors, such as infectious diseases. In an ideal national health care service, argues OHE, special provisions for the compensation of those damaged by vaccines should not have to be made because services for all the handicapped should be adequate. However, state compensation for vaccine-damaged people and their families may well draw attention to the poor provisions for the majority of brain-damaged people.

Improved living conditions, environmental health measures, new medicines and vaccination programmes, says the report, have reduced health risks in Britain from all known infectious diseases. In fact over the past 30 years, the number of child deaths from infectious diseases has dropped from about 10,000 to virtually none. Thus withdrawal of vaccination programmes which have outlived their usefulness should be considered.

Worldwide progress in smallpox immunisation has been so effective that in this country the risks associated with 'protection' outweigh the dangers of smallpox infection. The withdrawal of routine smallpox immunisation in 1971 by the NHS is supported by the OHE, although here again there has been much criticism of this action mainly because of the occasional reappearance of the disease.

All the same there appears to be strong evidence to indicate that there is a positive benefit to be derived from all the vaccines currently in use by the NHS, the only possible exceptions being BCG and pertussis vaccines.

The attention which pertussis vaccine has received was criticised by OHE as being over dramatised, particularly since the figures are not backed up with any definite evidence. The report says that there is no firm evidence to show that this vaccine's use causes more distress than benefit to the community as a whole.

OHE suggests that in view of the publicly voiced doubts as to the safety of pertussis vaccination priority should be given to a relatively swift action designed to restore confidence before other forms of immunisation are affected. An obvious option would be the temporary withdrawal of routine pertussis vaccine by the NHS, although this is not a recommendation of OHE. Recently the Department of Health and Social Security decided to continue pertussis vaccination while data necessary to make a critical review could be collected. The Institute of Biology endorsed this view when they submitted their views to the Royal Commission on Civil Liability and Compensation for Personal Injury.

Although it is unlikely that any major changes in vaccine policy will be made in the next few years the proposal to withdraw routine BCG vaccination is under discussion.

● AN idea for using the energy in the waves of the sea as a source of safe non-polluting power, developed by Dr Stephen Salter of Edinburgh University has attracted a Department of Trade grant of £60,000. As described by Dr Salter in an article in *Nature's* Energy Review earlier this year (*Nature*, **249**, 720; 1974) his system incorporates large steel vanes which harness the rolling motion of the waves, more efficiently than the up and down movement utilised by previous systems.

Apart from the Department of Trade, the Central Electricity Generating Board and a leading British concrete fabricator, Cementation Ltd., are also interested.

Also, Sir Christopher Cockerell, the inventor of the hovercraft, is a partner in a newly formed company, Wavepower Ltd, which is interested in the commercial application development of wavepower.

Astronautics in Amsterdam

from Angela Croome

Dr Kraft A. Ehricke belongs to the same generation of German-born space engineers and prophets as Werner von Braun and similarly helped to lay the foundations of the American space programme. It is not therefore too surprising that he retains an almost apocalyptic faith in man-in-space as bringer of solar energy (to supplement dwindling Earth resources) and grower of super crystals. That there are no limits to growth for life on this planet—only changes in pace and direction—was the theme of his keynote address to the 25th International Astronautical Congress, the annual rendezvous of the leading figures in the field, in Amsterdam last week. He urged growth and expansion in astronautics as a way of obtaining additional resources and accelerating the imperatives of international cooperation.

The note was not really in harmony, however, with the main tenor of the meeting. Programme scientists and project leaders today must have hard-headed arguments in justification of even low-cost automatic satellites. An all-day symposium on cost reduction in space operations is now a regular feature of the Congress, in fact. Dr Ehricke's industrial suburbs in orbit were not included in the programme.

The principal way of reducing the costs of space operations, of course, is to share them. The degree to which NASA has embraced this policy was heavily underlined at the Amsterdam gathering. Detente has certainly eased the way for American-Soviet space cooperation (where only a few years ago the 'space race' was an extension of the cold war) but the sight of Tom Stafford, captain of the American crew for next summer's Apollo-Soyuz rendezvous and docking project, and Alexei Leonov, the Russian crew leader giving a joint briefing on progress without interpreters gave credibility to the plan that has hitherto been lacking. Officially each country is contributing 50/50 to development, the 5 joint experiments, project management, even the command during the 2 days when the two capsules will become one; unofficially one learns that the exchange of information is all 'one way' and in particular the American space medicine experts responsible for crew fitness are unhappy at the lack of Soviet data.

There is only one American-Soviet flight scheduled at present and nothing definitely planned beyond. Next spring the two sets of senior negotiators (which includes the President of the Academy of Sciences on the Russian side) will consider possible further joint ventures, and Soviet participation in the reusable Shuttle programme for the 80s has been mentioned. Meantime Europe through ESRO is firmly committed to designing and building the Spacelab element of the shuttle programme which will substantially share the burden of the civilian sector of the programme with NASA. What is now being equally stressed is the virtue of having an integral European contribution to some of the promising planetary programmes of the next 10 years. A system of multiple spacecraft to collect and recover some soil samples from the surface of Mars would be substantially aided by having, for example, Europe build and operate one of the Mars descent vehicles. Similar arguments apply for a 1978 Venus probe in two parts and ESRO is studying a Jupiter mission for the 80s which would be on a joint contribution basis.

A note of caution was struck in the law sessions. The problems of liability in the case of a failure or an accident where several nationals are taking part in a common spacecraft will be formidable and there is no really adequate international court to decide possible space disputes.

Maltese conference cross over Caracas

from Wendy Barnaby, Malta

"It is", said Lord Ritchie-Calder, "the biggest smash-and-grab since the European powers at the Berlin Conference of 1885 carved up Black Africa." And his view of the Caracas United Nations Law of the Sea Conference was echoed by many of his fellow participants at Pacem in Maribus. The fifth meeting of this group, which can claim to be the most influential non-governmental organisation considering matters relating to the oceans, was held in Malta from September 9–13. Attracted to this discussion on the results of the Caracas proceedings were 64 people from 18 nations, the World Bank, the United Nations Environment Programme, the Commonwealth Secretariat and a number of non-governmental organisations. The statement which summarised the meeting's discussion was highly critical of Law of the Sea developments.

It was generally thought that the concept of a twelve nautical mile territorial sea plus a 200 nautical mile exclusive economic zone would eventually be accepted by the U.N. conference, possibly at its next session at Geneva in March 1975. Dr. Arvid Pardo, the original initiator of the U.N. Conference, argued that these concepts will not provide the necessary precision on the scope of national jurisdiction because rules determining the drawing of baselines, from which each zone will be measured, have not been agreed upon. Nor are they likely to be. Neither has agreement been reached on the relationship of the continental shelf to the economic zone. What will happen to those parts of the shelf which extend beyond 200 miles from the baseline?

As present technology makes exercise by states of their rights to expore and exploit the resources in the economic zone easy to the depth of 200 metres, it would be possible to imagine those states with extensive continental shelves taking advantage of the imprecision of baselines to push their economic zones out to the limits of their shelves. The inequalities this would entail seem too blatant to be borne.

This question of the inequalities built into the proposed system let loose another shower of criticism, this time from Dr. Sidney Holt, the Director of the International Ocean Institute in Malta. He pointed out that although the underdeveloped countries may benefit in the short term from the economic zone concept (on the argument that, even if it will make the rich richer, it will at least give the poor more than they have at the moment), there is no guarantee that their long-term benefit will be great. This is because the potential yield from the living resources within the economic zone is limited to double the present catch, while the potential of living resources much further out is one or two magnitudes greater. This mid-water life, which includes pelagic squid and the shrimp-like krill of the Antarctic ocean, needs special technology to harvest it. It is a reasonable bet that the developed countries will perfect this before the underdeveloped ones. If the latter are not to miss out in the future they should be pressing for international arrangements to enable co-operation with the developed countries in the utilisation of these resources.

A more immediate deficiency in the Caracas machinery was the inconsistency in terms of reference between the Conference's three committees. The second and third committees dealt with the ocean space including the water column, but the first committee was limited to the seabed. The result is that "the authority" which each committee was trying to set up within its own terms of reference will have ambiguous jurisdiction. Clearly this ought to be sorted out quickly at Geneva. And, just as clearly, the Conference should adopt the solution favoured by Pacem in Maribus: jurisdiction that will cover the ocean not just the seabed. The seabed cannot be dissociated from the water column in the sort of issues that will face the world community in its attempt to draw up a law of the seas.

ACCORDING to a remark often attributed to Mark Twain, "everybody talks about the weather, but nobody does anything about it". In the United States, where the federal government spends about $20 million a year on weather modification research (the actual amount depends on how you define the enterprise) and where the Department of Defense has recently admitted conducting a massive cloud seeding programme in South east Asia, Twain's remark is demonstrably untrue. Nevertheless, nine separate reports in as many years have all said that nobody is doing enough.

The latest in this series, a study conducted by the General Accounting Office (GAO), is no exception for it suggests that weather modification research is financially undernourished, badly coordinated and poorly focused. Such complaints have, of course, been made before for virtually every branch of science but in this case they merit some attention since for one thing the GAO has no axe to grind, and for another they are couched in language which, for the GAO, is uncharacteristically blunt.

A science born in 1946 when Vincent Schaefer and Irving Langmuir seeded rainclouds with pellets of solid carbon dioxide, weather modification moved briskly into commercial application—as much as 10% of the United States was under commercial cloud seeding operations in the 1950s—but it is still full of uncertainties. Even the Pentagon's rainmaking activities in Vietnam, for example, are generally regarded as having had only a marginal effect on rainfall there, and most weather modification experiments conducted in the United States have had mixed results.

Nevertheless, a number of organisations including the National Academy of Sciences and a Presidential advisory committee have argued during the past decade that a variety of weather modification techniques show sufficient promise for the federal government to initiate a centrally directed, adequately funded research programme.

But weather modification activities are now spread among some seven federal agencies, and the GAO has found that "an effective overall national weather modification program has not been established". Attempts to coordinate weather modification research, GAO continues, "have been somewhat disappointing", and the fragmentation of effort "has made it extremely difficult for agencies to conduct effective field research".

The GAO identified some $17.4 million in the federal budget for the 1974 fiscal year (which ended on June 30) for weather modification experiments, a level which falls dismally short of all the recommendations during the past decade. The National Academy of Sciences, for example, recommended as long ago as 1966 that $30 million a year could be wisely spent.

But the GAO's chief complaint (and, indeed, that of everybody else who has reported on the matter) is that no agency has been given the power to coordinate and manage an overall weather modification programme.

As an example, GAO singles out the National Hail Research Experiment, a $16.5 million project begun in 1972 which is designed to test various methods for eliminating or mitigating the effects of hail storms. Six different departments and agencies are taking part, with the National Science Foundation in a leading role.

Après moi . . .

Although GAO is quick to point out that the experiment has already yielded some promising results, it lists a number of cases in which agencies failed to meet their obligations, presumably because they accorded the project a low priority. The Department of Commerce, for example, failed to furnish radars and aircraft for the experiment in the first year, the Department of Defense did not supply helicopters as planned, and the National Science Foundation had to put up funds for a part of the project carried out by the Department of Agriculture.

Such problems, GAO reckons, would not have arisen if a national research programme were established and a single federal agency were given responsibility for implementing it.

● THE supplementary agenda of the current session of the United Nations contains a resolution, proposed by the Soviet Union (in a letter of August 7, 1974), urging "The prohibition of action to influence the environment and climate for military and other purposes incompatible with the maintenance of international security, human wellbeing, and health." Not surprisingly, this resolution is receiving much favourable publicity in the Soviet press. While one must applaud the laudable sentiments of this resolution, a certain doubt nevertheless arises; since the Soviet Union is one of the chief exponents of climatic manipulation (for peaceful purposes, be it understood), is there not perhaps the danger that such projects, however beneficial to the country concerned, may result in the deprivation of others, that one nation's plenitude may result in another nation's deprivation.

Apart from grandiose schemes for climatic manipulation in the distant future, such as their longstanding scheme to divert southwards those rivers which at present discharge uselessly into the Arctic Ocean and the creation of artificial glaciers in the deserts of Soviet Central Asia, alarming scheme are proposed in an imaginative survey *Life in the Twenty-First Century*, compiled in 1957. These include the damming of the Bering Straits and the pumping of warm water from the Pacific to the Arctic Ocean, so as to change the climate of Kamchatka, North-East Siberia and the Arctic Basin. Similarly, it is proposed to join Sakhalin Island to the Soviet mainland, and, by pumping warm water from the Kuro-Siwa current through the dam into the Tartar Strait, to warm the Sea of Okhotsk. Naturally, in this panegyric, undesirable side-effects are minimised—there is no more than a joking reference to the Japanese skiing enthusiasts who no longer (in AD 2007) have any snow.

At present, the principal efforts of the Soviet Union are aimed at what *Pravda* calls "hail-prevention" — in reality, hail-making. Research in this field was begun in the late 1960's at the Institute of Alpine Geophysics at Nalchik in the North Caucausus. Potential hail clouds are observed by radar, and then seeded by lead iodide from a rocket fired into the cloud's centre. Although the method appears to be reasonably successful—provided that the hail cloud is spotted in time—it does result in heavy hail falls in surrounding areas. By 1971, the technique was considered sufficiently reliable to form part of the Soviet aid programmes to Argentina, the Lebanon, Hungary and Yugoslavia.

EPA firm on pesticides ban

from Colin Norman, Washington

RUSSELL TRAIN, Administrator of the Environmental Protection Agency, last week held firm on his decision to halt production of the widely used pesticides aldrin and dieldrin, pending the outcome of public hearings on charges that the chemicals may be carcinogenic to man.

The decision is an attempt to establish an important concept in the regulation of environmental chemicals—that possibly dangerous substances should be removed from the market until they are found to be safe, rather than being kept in use until they are definitely found to be hazardous.

That concept is already well established in the regulation of food additives in the United States, thanks to a legal requirement that chemicals cannot be added to food if they are found to increase the incidence of cancer in test animals, but attempts to apply it to the regulation of other environmental chemicals have so far met with mixed success. Moves against aldrin and dieldrin were made by the EPA as long ago as 1971, when the agency issued an order to cancel the pesticides' registration—which was tantamount to banning their use. That order was based on findings that the pesticides increase the incidence of liver tumours in mice when fed to them in relatively high doses. But the Shell Chemical Corporation, sole manufacturers of aldrin and dieldrin, appealed against the order, and the appeal has been making glacial progress through the courts and EPA's administrative machinery ever since.

Meanwhile, evidence has accumulated that aldrin and dieldrin raise a variety of tumours in at least five strains of mice and possibly rats as well. Their residues can be found in the tissues of virtually every man, woman and child in the United States, and a survey conducted last year by the Food and Drug Administration found the pesticides in 83% all dairy products, 88% of garden fruits, and 96% of meat, poultry and fish.

On the basis of that evidence, EPA privately asked Shell to stop producing aldrin and dieldrin, at least until the appeals process on the cancellation order has been exhausted. But Shell refused, and announced that it would produce about 10 million pounds of the pesticides this Autumn for use in 1975, knowing full well that if EPA's cancellation order is upheld next year it would be virtually impossible to call in the pesticides which had already been sold. Train therefore exercised emergency authority in July to order Shell to stop making the pesticides. Shell appealed against that ruling, however, and demanded a public hearing on the matter. The hearing took place in August and early September before Administrative Law Judge Herbert L. Perlman, who upheld Train's order on September 20, as a result of which Train last week refused to reconsider his decision. Shell officials immediately announced that they will take their case to the federal courts.

There is no dispute over the fact that aldrin and dieldrin increase the incidence of liver tumours in mice—in fact, Shell representatives even conceded that point during the public hearings—but there is a dispute over the relevance of that fact for humans.

According to Dr Don Stevenson, Director of Shell's Tunstall Laboratory, for example, five separate criteria must be met before there is sufficient evidence of human carcinogenicity. There must be a higher incidence of tumours in exposed animals, tumours should develop in more than one species, there is adequate proof that the development of the tumours is related to the test compound, the animal has proved to be an adequate model for extrapolating to man, and that there is proof of at least one incidence of cancer in man related to the test compound.

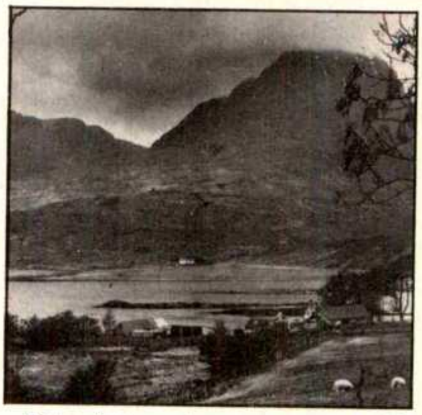
As Train pointed out last week, such criteria would be impossible to meet since cancer in man often takes several decades to develop after the initial exposure. Both Perlman and Train therefore concluded that there is already sufficient evidence of carcinogenicity in mice to suggest that aldrin and dieldrin pose a high risk to man.

Meanwhile, the appeals process for EPA's original cancellation order against the pesticides grinds on. Public hearings have been dragging on since August 1973, and according to Train, they will continue into the "indefinite future". It is worth noting, however, that although the production ban in theory does not prejudge the cancellation issue, Perlman is sitting as hearing examiner for the cancellation hearings as well as for the hearings into the production ban. His 109-page opinion on EPA's production ban represents such a severe indictment of the pesticides that it seems unlikely that he will ultimately give them a clean bill of health.

AFTER the good news about Drumbuie, the bad news about Kishorn. The Secretary of State for Scotland, Mr William Ross, has approved an application by the Howard/Doris partnership to build concrete gravity-type oil production platforms at the Loch (pictured). On the face of it the Kishorn site seems to satisfy none of Mr Ross's criteria for a suitable site; that it must have access to existing facilities, that it can draw on existing labour sources and that it can make use of existing infrastructure.

By giving this decision Mr Ross has brought down on himself a hail of criticism, not only from those who successfully opposed the development at Drumbuie, but also from those who felt that Drumbuie would be the lesser of two evils. As the site is even more remote than Drumbuie from existing rail and road services, all materials and supplies are to be shipped across the loch from the nearest rail link, which will involve an additional development at this point.

But giving his reasons for the decision, Mr Ross emphasised that the very remoteness of the site was a point in its favour. Also, the site will employ a smaller workforce than that proposed at Drumbuie. Obviously a major factor in the differing decisions is the National Trust land involved at Drumbuie, and the fact that far fewer local residents were likely to be affected.



But what must worry many people is the fear that this is only the beginning. This decision has been made despite the recent publication of the Scottish Development Department's Coastal Planning guidelines which designate the West Highlands coast as a conservation zone. Mowlem/Taylor Woodrow who lost the site at Drumbuie, are unlikely to rest under this decision and will no doubt look for a site nearby. As necessary infrastructure is developed, the economic advantages of allowing more sites to use it will no doubt be cited.

A few crumbs of comfort may be gathered in the appendices to the Secretary of State's memorandum. Twelve years from now, the site must be cleared completely of all buildings, and restored to its original condition, with replanting if necessary. Optimists can book their holiday now.

news and views

Genetic engineering with viruses

A SIGNIFICANT development in the field of genetic engineering is described in an article by Drs Noreen and Ken Murray in this week's issue of *Nature* (page 476) They have been able to isolate a mutant of bacteriophage λ which can replace the normally used plasmid in experiments designed to construct new hybrid DNA molecules

These experiments are very simple in principle and (in brief) involve mixing together in a test tube the donor DNA molecule (the gene to be studied), the receptor (the plasmid) and a 'DNA cutting' enzyme, the restriction enzyme *Eco*RI The cutting enzyme breaks the circular double stranded plasmid at a single point converting it into a linear molecule But it does more than this The enzyme cuts DNA slightly asymmetrically leaving four nucleotides projecting at each end These 'sticky ends' can pair up by forming Watson–Crick base pairs with similar sticky ends formed on the donor DNA molecule by the cutting enzyme. Thus a linear hybrid plasmid is formed which can circularise To stabilise the new hybrid plasmid another enzyme, DNA ligase (a 'joining' enzyme), is added to reform the covalent bonds broken by the restriction enzyme The success of such experiments, which have now been carried out using staphylococcal DNA, *Xenopus laevis* ribosomal genes and *Drosophila* DNA as donor, depends on there being a single site for the restriction enzyme on the plasmid The problem in phage λ is that there are five sites

The Murrays' work involves a brilliant series of genetic and biochemical manipulations to construct a mutant phage λ with one or two sites for the restriction enzyme *Eco*RI Then, in subsequent experiments, restriction fragments of these various mutant phage λs were joined together *in vitro* to form hybrid molecules with a deletion in a non-essential part of the phage These new molecules were isolated as biologically active phage λ after infection of *Escherichia coli* By this means the single-site mutant was converted to a bacteriophage with a gap in it leaving room for an incoming piece of DNA The authors were able to insert the genes specifying the biosynthesis of tryptophan in *E coli* (the *trp D* and *E* genes) into this receptor phage λ They imply that they hope to extend this work to study the expression of the *trp* genes, for which phage λ is an ideal host (and has significant advantages over a plasmid host), as well as to study the insertion of DNA from higher organisms

Many readers of *Nature* will know that there is some controversy among scientists about the ethics of performing genetic engineering experiments (see *Nature*, **250**, 279, 1974) Part of the concern is about the nature of the particular *E coli* plasmid used in these experiments which confers on the *E coli* resistance to the antibiotic tetracycline Clearly, if one wishes to introduce foreign, and possibly hazardous DNA, it is advantageous to use microorganisms susceptible to antibiotics Dr K Murray, in a recent lecture to the Biochemical Society, has pointed out that phage λ is much safer than the plasmid It is sensitive to antibiotics and only infects some strains of *E coli* There is also evidence that the laboratory strains for which λ is infective do not establish themselves in the human gut It is thus improbable that hybrid molecules (of whatever type) could be pathogenic

G G BROWNLEE

Some myths about heritability and IQ

THINGS have gone too far Estimates of the heritability of IQ reported in *Nature* have assumed a distinctly bimodal distribution First, Eaves and Jinks[1] decried my data on the heritability of scholastic aptitudes[2] as a failure to detect the high heritability of IQ In a sample of urban school-age twins, I found that black and economically disadvantaged children from both races seemed to have lower heritabilities for verbal and quantitative aptitudes than white and middle-class children In some cases heritabilities were not calculable, because the correlations of opposite-sex twins were slightly larger than those of same-sex twins Now, Schwartz and Schwartz[3] proclaim zero heritability for IQ as the obvious conclusion to be drawn from the same study and from Kamin's iconoclastic efforts (Invited Address, Eastern Psychological Association, Washington DC, March 1973) I beg to differ with both extremes and to argue against any simple conclusions on the issue

The notion that there is a single answer to the question "What is the heritability of IQ?" is patently false Heritability estimates vary according to what skills are measured as intelligence, by how they are tested, by the age at which abilities are measured, and by the genetic and environmental composition of the population tested To claim that there is a single estimate for the genetic contribution to human abilities is to deny, first, half a century of evidence on differential abilities Some aspects of intelligence, such as vocabulary, have consistently higher heritability estimates than, say, numerical reasoning Even if one concluded, with Burt, that general intelligence (*g*) is more important than specific abilities, different tests of *g* will still yield different heritability estimates[5]

Differences in heritability estimates across abilities and tests are exceeded only by differences across age groups and populations Bayley Infant Intelligence Test scores for the first two years have been found to have generally lower heritabilities than later scores Even early heritabilities vary enormously from 0 2 to 0 4 for siblings (McCall, American Psychological Association, Honolulu, September 1972), 0 75 for some twins (Nichols and Broman, preliminary report of the Collaborative Perinatal Study, 1973); and 0 3 for other twins[6] Across populations, Nichols[7] reported a significantly lower correlation for black than white siblings on the Stanford–Binet test at age 4 Another twin study (Scarr-Salapatek, Katz, and Barker, *Black and White Twins*, in preparation) found lower heritabilities for adolescent black and white twin pairs on each of four tests of cognitive abilities and for the first principal component from the four tests These data are presented in Table 1 Not only are

Table 1 Heritability estimates for four tests of cognitive abilities in two adolescent twin populations

Test	Black h^2* ($N=160$ pairs)	White h^2 ($N=211$ pairs)
Raven Standard Progressive Matrices	0.59	0.87
Peabody Picture Vocabulary Test	0.28	0.49
Columbia Test of Mental Maturity	0.42	0.58
Revised Test of Figural Memory	0.61	0.71
First Principal Component	0.48	0.63

*$h^2=2\,(r_{i\mathrm{MZ}}-r_{i\mathrm{DZ}})$, where $r_{i\mathrm{MZ}}$ is the intraclass correlation for monozygotic twins and $r_{i\mathrm{DZ}}$ for dizygotic twins.

heritabilities lower for black children on all four measures, the heritabilities are not uniformly 0.80 or 0.00 in either group.

If one observes an array of data from studies of related and unrelated people, the IQ scores of those more closely related generally correlate more highly than those more distantly related. This is undeniable. The view from afar reveals important regularities in the results. If the proponents of a uniformly high heritability would look more closely, however, at the constellation of human differences, they would find important inconsistencies as well.

A recent reanalysis of data on the heritability of Stanford–Binet IQ scores questions the very high heritability estimate that Eaves and Jinks[1] propose. Jencks[8] reanalysed US family correlations and obtained heritability estimates between 0.45 and 0.60, far below the 0.8 claimed by Burt[9], Jensen[10], Eaves and Jinks[1], and Herrnstein[11]. If Burt's data are included, the heritability estimates rise, in large part because his observed (?) correlations fit genetic expectations so perfectly. As Jensen[12] has recently shown, however, there are serious inconsistencies in Burt's reports. If one relies primarily upon Burt's data, one could be grossly misled as to the proper range of heritability estimates.

A reanalysis of Jencks's reanalysis[13] suggests that the best fit to the US data is a heritability of about 0.68. But Eaves and Jinks ignore Jencks's major point: that the data from parent–child, twin, sibling, and adopted child pairs do not fit any simple model! There are sufficiently serious disagreements that forcing the data into a biometrical model is to risk a nonsense solution—however mathematically elegant.

In addition, twin data on which the proponents of high heritability rely have been shown to overestimate heritability values[14], presumably because twin environments, particularly those of monozygotic twins, have less variability (pre- and post-natally) than the environments of ordinary siblings. The variance between monozygotic co-twins is further reduced by genotype–environment covariances and interactions. There has not yet been a serious test of the magnitude of covariance and interaction effects on human intelligence. Although Jinks and Fulker[15] suggest a test for interaction (the correlation of half the sum and half the difference between IQ scores of separated monozygotic twins), their barely significant results are based on very small samples.

Further evidence on the exaggeration of heritability values comes from Morton[16]. He revived Wright's[17] reanalysis of Burks's[18] adoption study. Burks's faulty path analysis model yielded a heritability estimate for general IQ (in white California adoptees) of 0.75 to 0.80. Wright's more adequate path model resulted in a heritability of 0.50 from the same data. Morton's additional calculations for sibs and foster sibs gave a heritability solution of 0.52.

The idea of zero heritability strikes most geneticists and biologists as odd (Fuller, presidential address, Behavior Genetics Association, Minneapolis, June 1974). How can there be any biological characteristic for which genetic variability makes no contribution to phenotypic variability? As Morton[16] remarked, " the experience of biometrical genetics [is] that a trait heritable at its extremes has never been found to have zero heritability within the normal range" (page 257). Certainly IQ is affected by many single gene and chromosomal anomalies. There are four possibilities to explain a finding of zero heritability: first, the measurement of the phenotype is invalid or unreliable, second, there is no genetic variability underlying the phenotype, third, all genotypes are functionally equivalent in producing the phenotype, or fourth, other effects overwhelm genetic variability. The first two possibilities for the heritability of human abilities can be dismissed out of hand. The third is what Schwartz and Schwartz[3] want to argue. There is simply too much evidence to the contrary, however, from studies other than those that they and Kamin would dismiss. Though every human family study may be criticised on one or more methodological grounds, their flaws are varied and non-overlapping. The weight of evidence suggests that genetically related persons are more similar intellectually than unrelated persons, whether they are reared together or apart. The microscopic examination of research flaws has blinded some critics of heritability to the obvious pattern of results.

The fourth is what Henderson[19] and I[20,21] argued for some populations. Other effects can include rearing conditions, growth patterns at a particular age, the skills tested, and many unspecified conditions that will affect sources of variability. The negligible and low heritabilities reported for aptitude scores among disadvantaged, school-aged twins in Philadelphia[2] were a function of the environments sampled, the tests, the populations, and possibly the age group. The point is that genetic differences did not seem to contribute much to variability in aptitude scores in those populations. Different tests, ages, populations, and cohorts may well yield other results. Since these tests are very widely used to make life decisions for children, however, their low heritabilities have considerable implications.

There is no single answer to the question "What is the heritability of IQ?" because it is a pseudo-question. Heritability estimates range between 0.00 and 0.9 in reported research, but most values are in a middle range. An accurate view of genetic contributions of differences in IQ cannot be obtained from the proponents of extreme positions. In fact, the best view of human variability can be obtained by getting off the political platform and out there to study families. With the notable exception of Arthur Jensen, not many advocates of high or low heritability are adding to our store of knowledge about human intelligence.

SANDRA SCARR-SALAPATEK

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Origin of metalliferous sediments

FOR reasons which are largely historical and political rather than scientific, the first specific phenomenon likely to come to mind at any general mention of ocean mineralisation is that of manganese nodules No doubt there is some justification for this emphasis, for in spite of the many unsolved problems relating to both their collection and processing, nodules will almost certainly be the first deep sea minerals to be exploited commercially But nodules are not the whole story of marine mineralisation, nor, as Bullard (*Endeavour*, **33**, 80, 1974) has pointed out, will they necessarily prove to be the most important part of it in the long run Nodules are attractive because they are discrete conglomerations of a few metals in a particularly concentrated form, but in both economic and scientific terms they are likely to be rivalled increasingly by sediments containing unusually high proportions of similar elements

The story of the discovery of metal-rich sediments begins in 1948 when Swedish workers on the research vessel Albatross discovered a region of abnormally high temperature in the Red Sea just north of latitude 21° N About 15 years later, more detailed investigations of water temperature and salinity were carried out in the same region by teams from the United States, Germany and Britain, and as a result of the discoveries made then, a further study of both water and underlying sediments was undertaken in 1966 by the research vessel Chain from the Woods Hole Oceanographic Institution These expeditions found three deep basins containing hot water with a salinity about seven times greater than that of normal Red Sea water The largest of these 'brine pools' was the Atlantis II Deep (about 15 km long by 5 km wide), although it became clear that this is not the deepest of the brine-filled basins when Backer and Schoell (*Nature phys Sci*, **240**, 153, 1972) reported the discovery of 13 more brine pools by the German ships Wando River and Valdivio

But if the water in the Red Sea deeps is remarkable, equally so are the sediments beneath them For whereas the normal sediments in the central trough of the Red Sea are light-coloured marls, those in the brine pools were found to comprise yellow, red, brown and blue layers enriched in such metals as iron, manganese, copper and zinc The enrichment seems to be high but quite variable In 1969 Bischoff (in *Hot Brines and Recent Heavy Metal Deposits in the Red Sea*, Springer-Verlag, 1969) said that the high grade sediments in the Atlantis II Deep contain an average of 3 4% Zn and 1 3% Cu after removal of salt and water But Tooms (*Endeavour*, **31**, 113, 1972) has quoted a more recent survey by a commercial company which led to average estimates over a much wider area of 6% Zn and "several per cent" Cu Even more recently, Backer and Schoell have claimed that some facies contain 50–65% iron, that the average manganese concentration in the Chain Deeps is 30–40% and that some sulphide layers in the Atlantis II Deep contain 20% zinc, although they admit that "continuously changing facies conditions" reduce the average base metal content to a few per cent

But although quoted metal concentrations differ, there is complete agreement that enrichment has taken place, and this inevitably raises the question of how and under what circumstances it occurred The Red Sea is widely believed to be an embryo ocean, and the deeps containing hot brine are closely associated with the central trough The connection between metallogenesis and seafloor spreading is thus unmistakable—but is metal enrichment a feature only of spreading in its early stages or does it occur whenever and wherever spreading takes place? The thick salt deposits covering a large part of the Red Sea depression must be the ultimate source of the salt in the brine pools, and the brine pools are closely associated with the enriched sediments beneath Does this mean that metal enrichment only takes place in relatively closed oceans conducive to the formation of salt deposits by repeated evaporation, or is the presence of abundant salt in itself an unnecessary condition?

These questions cannot yet be properly answered But there is now abundant evidence for the existence of metal-rich sediments on the East Pacific rise and other ocean ridges Such sediments were apparently known to Revelle (*Publs Carnegie Instn*, **556**; 1944) thirty years ago, although modern studies can probably be said to date from the geochemical work of Bostrom and Peterson (*Econ Geol*, **61**, 1258, 1966) following the discovery of the critical part played by ocean ridges in global tectonics In this and numerous subsequent reports, Bostrom and his colleagues and others have shown that metal enrichment (but not apparently to economic concentrations) does indeed take place along ridges in late stages of spreading, and Deep Sea Drilling Project results have indicated that such sediments are not uncommon at the base of sedimentary columns throughout the ocean basins

The general circumstances in which metalliferous sediments are likely to form are thus at least partly understood, but precisely how they form is still an unsolved problem Nevertheless, it is becoming increasingly clear that hydrothermal processes are involved in some way In the case of the Red Sea, for example, it may be presumed that the salt in the brine comes from the existing salt deposits, but the water must have acquired its temperature from the upwelling magma at the central rift It is then reasonable to suppose that, during contact with the magma, the water also acquired metallic components which later precipitated out as metallic compounds to form the observed sedimentary ores

In fact, water may well penetrate to substantial depths (possibly several kilometres) in the new oceanic crust at ridge axes, thus leaching a considerable volume of rock Both Corliss (*J geophys Res*, **76**, 8128, 1971) and Spooner and Fyfe (*Contr Mineral Petrol*, **42**, 287, 1973) have proposed models in which sedimentary metal enrichment could occur by this mechanism, and Lister (*Geophys J*, **26**, 515, 1972) has used deep hydrothermal circulation to explain the unexpectedly low average heat flow at ridge crests and the high scatter of heat flow values at ridge crests and flanks Piper (*Earth planet Sci Lett*, **19**, 75, 1973) and many others have offered geochemical evidence, and on page 473 of this issue of *Nature*, Anderson and Halunen present combined geophysical and geochemical data which give further support As Lister (*Eos*, **55**, 740, 1974) has rightly suggested, what is required now is a commitment to test the hydrothermal circulation hypothesis more directly by deep sea borehole measurements

PETER J SMITH

Cell culture pharmacology

from Mary Dawson

THE action of drugs on cultured vertebrate cells was the subject of the autumn meeting of the British Society for Cell Biology, held at the University of Strathclyde, Glasgow, on September 19 and 20, 1974 Most of the experiments described had one of four main objects—to increase knowledge of the cells being studied by using known compounds as tools, to elucidate the mode of action of drugs where this was unknown or partly known, or to design *in vitro* tests which would either provide knowledge about new drugs under development or would form a basis for choosing the best drug from a range of possibles for any one patient Of the papers presented more had been engendered by cancer research than by any other single disease area This is a fair representation of cellular pharmacology work since it is logical and practicable both to study the effects of compounds on normally and abnormally proliferating cells and to use compounds to study differences between such cells

The Searle Lecture was delivered by L J Tolmach (Washington University) who spoke of his group's work on the enhancement of radiation effects on proliferating cells by various drugs used separately or together He emphasised, as did several subsequent speakers, the relative ease of carrying out statistically satisfactory numbers of replicate experiments in cell culture, and of automating additions and removals of drugs Drug–radiation interaction was discussed also by A H W Nias (Belvidere Hospital, Glasgow) and papers on specific groups of compounds were presented by A M Creighton (Imperial Cancer Research Fund) and B J Phillips (Chester Beatty Research Institute)

The papers of R I Freshney (Beatson Institute, Glasgow) and J A Dickson (Royal Victoria Infirmary, Newcastle-upon-Tyne) dealt with the design of cell culture tests for measuring human tumour sensitivity to drugs, and the place of such tests in cancer therapy They described the uses and limitations of such tests, and factors influencing the results, pointing out that a laboratory LD_{50} end point still meant 50% of surviving cells too

A further group of four papers was on the subject of cardiac glycosides and electrophysiology experiments These were by J F Lamb (University of St Andrews) and W F Dryden *et al* (University of Strathclyde) There were two papers on anaesthetics and related compounds by L J Erkell (Goteborg) and A G Macdonald (Aberdeen) The latter paper led to comment on the validity of using non-mammalian material to investigate clinical problems, as did the paper of D Brown, M Balls and J Pryor (University of East Anglia) who chose amphibian cell cultures for their relative functional longevity The symposium title had been deliberately widened from "human" to "vertebrate" cells to generate discussion on the possibility of errors due to species difference on extrapolating from various types of *in vitro* tests to clinical situations There was indeed much discussion on choice of culture material

The action of parathyroid hormone on explanted bone rudiments was discussed by P J Gaillard (University of Leiden) in relation to hyperparathyroid conditions, and P J Hornsby and M J O'Hare (Chester Beatty Institute) described functional activity in cultured adrenal cells The extraordinary biological properties of the cytochalasins were discussed by S B Carter (Imperial Chemical Industries, Macclesfield) who indicated their value in examining cellular processes as did M de Brabander (Janssen Laboratories, Beerse)

Further areas where cell culture study was relevant to finding information about the compounds or the cells were reported in the papers on prostaglandins by M Adolphe (Institute of Pharmacology, Paris), on *in vitro* antibody production by G Harris (Kennedy Institute, London), on bacitracin by M Dawson (University of Strathclyde) and on colchicine and microtubules by D Wheatley (University of Aberdeen)

Mystery molecule in Japan

from Sydney Shall

THE Japanese have taken poly(ADP–ribose) to their heart Since much of the progress in this area of molecular biology comes from Japanese laboratories, this is understandable The occasion of the first Oji International Seminar at Tomakomai, Hokkaido, was used by Professors Osamu Hayaishi (University of Kyoto) and Takashi Sugimura (University of Tokyo) to organise the third international meeting on poly(ADP–ribose) and the ADP–ribosylation of proteins (September 10–12). This very young field has progressed steadily over the last five years, but without success so far in two major respects a simple method for measuring poly(ADP–ribose) in intact cells, and unequivocal evidence of its physiological function are both lacking

This unusual chromosomal polymer has a composition almost identical to that of poly(A) but does not have a nucleic acid structure It is made by a chromosomal enzyme for which NAD$^+$ is the sole substrate NAD$^+$ metabolism in cancer cells is unusual and poly(ADP–ribose) is thought to have some role in DNA replication and cell proliferation

The highlights of the meeting included progress in the purification (5,000 times) of the poly(ADP–ribose) polymerase by Ueda and Hayaishi (University of Kyoto) The partially purified enzyme still requires both DNA and histones and makes short chains The enzymes that degrade the polymer have been isolated and purified by L Burzio (Rockefeller University, New York) from testis, and by M Tanaka, M Miwa and T Sugimura (University of Tokyo) from liver, thymus, *Physarum polycephalum* and from tobacco cells in culture

There was considerable discussion following the paper by H Hilz (University of Hamburg) as to whether the polymer is covalently attached to chromosomal protein Hilz demonstrated that at least some of the polymer is covalently bonded to protein

The most interesting report was the description by T Kanai and T Sugimura (Tokyo) of the preparation of antibodies to poly(ADP–ribose) They showed that with these antibodies a radioimmunoassay for poly(ADP–ribose) will be possible The antibodies seem to be specific and do not cross-react with DNA, RNA or poly(A) In addition, they have detected anti-poly(ADP–ribose) antibodies in the sera of human patients with the autoimmune diseases, especially in systemic lupus erythematosus H Hilz has estimated the amount of poly(ADP–ribose) in intact animals by an extremely carefully controlled, isotope dilution technique Three important conclusions emerge the polymer exists in intact cells, the amount of polymer was estimated in adult rat liver to be 5 25±0 09 nmol of ADP–ribose residues per mg DNA, no striking difference in amount was found between adult rat liver, neonatal rat liver and Ehrlich ascites carcinoma cells But in isolated nuclei there is a substantial difference in the content of mono(ADP–ribose) residues between adult and neonatal liver

Other examples of ADP–ribosylation of proteins are very well authenticated The modification and alteration of DNA-dependent RNA polymerase by ADP–ribosylation after T4 phage infection of *Escherichia coli* was clearly demonstrated by W. Zillig (Max-Planck-Institut, Munich) and C G Goff (Medical Research Council, Cambridge) ADP–ribosylation of mammalian ribosomal elongation factor II by diphtheria toxin was dissected by C Edson, K Ueda and O Hayaishi (University of Kyoto) and E S Maxwell (National Institutes of Health, Bethesda) Both laboratories have so far failed to identify the ribosyl–protein bond in modified elongation factor But Maxwell showed the presence of an unusual amino acid in unmodified elongation factor II to which the ADP–ribose was apparently attached by the diphtheria toxin Finally, K Yoshihara (University of Nara, Japan) adduced evidence that the recently described chromatin-bound Ca^{2+}, Mg^{2+} endonuclease is itself ADP–ribosylated

The mysteries of poly(ADP–ribose) are steadily yielding to systematic attack The elucidation of its function will radically alter our picture of the biology of the cell nucleus

Fish out of phase

from Marian Dawkins

ANIMALS often need to distinguish between stimuli which arise from their environment (which includes other animals) and those which are caused by some action of the animal itself. An apparent movement of an object or a sound, for example, may require a completely different response depending on whether or not the animal has produced that movement or that sound by its own behaviour, even though the pattern of stimulation reaching the animal's sense organs may be extremely similar in the two cases.

Although the ways in which animals make this distinction are many and varied, a recently described example in a species of electric fish by Scheich (*Science*, **185**, 365; 1974) is of particular interest as it illustrates how the distinction can be made even in a situation where the animal must distinguish between two similar and simultaneous wave forms.

Scheich studied a species of fish, *Eigenmannia*, in which the electric organ has a regular wave-like discharge, enabling the fish to locate objects by detecting disturbances in the resulting field. When two *Eigenmannia* which are discharging their electric organs at similar frequencies come close enough together, they both unerringly adjust their frequencies in opposite directions so that their frequencies are no longer similar and the danger of interference to their object-detecting systems is avoided.

Conveniently for experimental study, a fish will also alter its frequency if stimulated through an electrical dipole in its tank at a frequency near its own. Within wide limits of absolute frequency, the fish will correctly alter the frequency difference between its own discharge and that of the applied stimulus to maintain a difference. In order to do this the fish must be able to detect how near its own frequency is to that of the applied stimulus and to have information on whether it is lower or higher.

A possible, although cumbersome way of doing this would be to have an array of frequency-tuned filters and for the fish to respond whenever two filters near enough in frequency were active together, provided it had some way of telling which frequency filter was responding to its own discharge. In fact, as Scheich demonstrates, the fish's solution is much simpler and his theory accounts more satisfactorily for the fish's ability to recognise the sign of the frequency difference whatever the fish's own frequency within that range.

The analysis is done by sensitivity to the beating of the two signals in the electric field—that is, the coming into and out of phase of the two waves of slightly different frequency. The frequency with which the two waves come into phase with one another (like the frequency of beat notes with two sound waves) is the difference between the two frequencies and will be independent of absolute frequency.

If the electric organs discharged sinusoidally there would be ambiguity as to which signal belonged to which fish, but the discharge is not sinusoidal: the wave shape is rich in harmonics and is clipped in one polarity. By being sensitive to the peculiarities of beat patterns produced by the interaction of two such oddly shaped waves, the fish can tell whether the interference is above or below it in frequency as well as how near in frequency. By ingenious experiments in which the fish's own output was converted artificially into a sine wave, Scheich found that under these circumstances, the fish was completely unable to distinguish the sign of the frequency difference between its own output and a sinusoidal applied stimulus. Only when its own output was allowed to resemble more closely the normal discharge was its normal behaviour restored. Scheich also found neurones in the fish's midbrain which responded to differences in frequency in such a way that they could directly provide the input into the electric organ.

Thus the fish is able to obtain information about how near its own frequency another fish is discharging and whether the other is above it or below it in frequency, not by analysing constituent frequencies, but rather by beat analysis in the time domain. It is interesting that a similar mechanism is postulated by some theories of hearing.

RNA molecules in initiation of protein biosynthesis

from Alan E. Smith

TEN years ago the main interest in protein biosynthesis was the establishment of the genetic code by translating synthetic polynucleotides of known composition or sequence in bacterial cell-free systems. Then it seemed reasonable to suppose that any ribosome can attach to almost any RNA molecule and translate it into polypeptide. This view is now known to be totally naive. The initiation of protein synthesis is a highly complex interaction between a ribosome, the initiator tRNA, and the specific ribosome-binding site on the mRNA. Several protein factors which also play a part in this process have various enzymatic functions: dissociating the ribosome into subunits, binding the initiator tRNA and binding the appropriate mRNA molecule. It now seems that in addition to the protein initiation factors, there are RNA molecules involved.

For some time, Heywood and his colleagues have been studying the specificity of mRNA translation in heterologous cell-free systems and have concluded that a specific mRNA can only be translated in a heterologous ribosome if a specific homologous initiation factor (designated IF_3) is present. Thus the synthesis of myosin on, say, erythroblast ribosomes requires initiation factors obtained from muscle ribosomes (*Biochemistry*, **11**, 2061–2066; 1972). When they further fractionated red muscle initiation factors required for the synthesis of myoglobin and myosin in a reticulocyte cell-free system, Heywood, Kennedy and Bester (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 2428–2431; 1974) found that two activities are present: one specifically stimulates myoglobin synthesis and the other stimulates myosin synthesis. In addition to these two proteins, an RNA molecule was isolated from the IF_3 preparation and this seems to inhibit the initiation and translation of heterologous mRNA molecules. Bogdanovsky, Herman and Schapira (*Biochem. biophys. Res. Commun.*, **54**, 25–32; 1973) have previously shown that a similar RNA molecule of molecular weight 11,000 is present in preparations of initiation factors from rabbit reticulocytes and that the RNA is required for the synthesis of globin *in vitro*.

The presence of a small RNA initiation factor may corroborate the observations of Reichmann and Penman. They found that the base analogue 5-azacytidine inhibits protein synthesis in HeLa cells with a half life of 90 minutes (*Biochim. biophys. Acta*, **324**, 282–289; 1973). The inhibition seemed to occur at the initiation of protein synthesis and was suppressed by actinomycin D. Earlier studies had established that the half life of mammalian mRNA is very long compared with the response to aza-cytidine, and this suggested that an RNA molecule other than mRNA or rRNA, which is rapidly metabolised *in vivo*, is involved in the initiation of protein synthesis. The authors then showed (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 2678–2682; 1973) that the putative RNA species accumulates in cells subjected to inhibition of protein synthesis by, for example, cycloheximide or starvation of an essential amino acid, and cell-free systems prepared from such cells show a greatly enhanced ability to initiate new polypeptide chains.

The evidence available to date indicates that the RNA species involved

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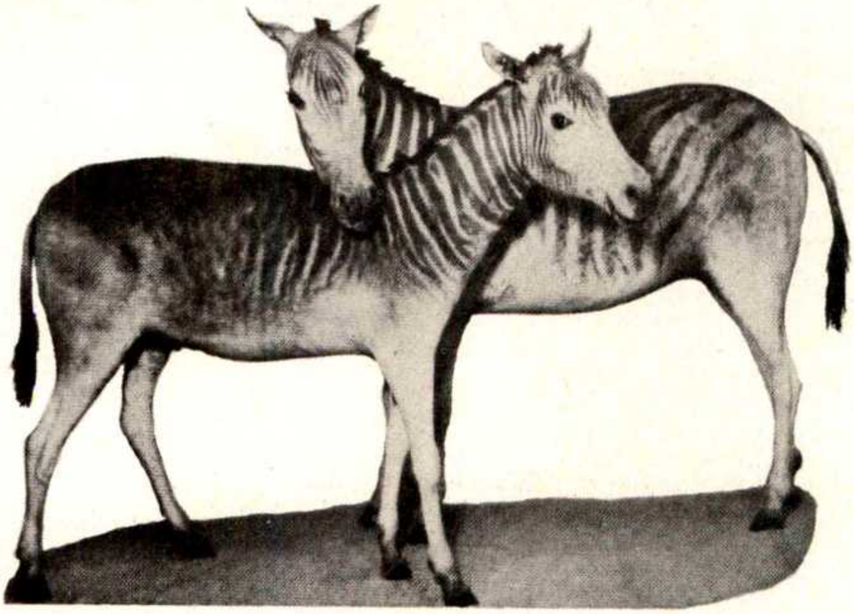
in initiation is not rRNA. Probably, therefore, the phenomenon described above is not related to the small 2S RNA species recently reported in *Drosophila* ribosomal RNA (B. R. Jordan, *FEBS Lett.*, **44**, 39–42; 1974) since this seems to be a cleavage product of 26S rRNA maturation. It is possible, however, that the mode of action of the small stimulatory RNA in eukaryotes resembles that proposed for the 3′-OH end of 16S RNA in bacteria. Shine and Dalgarno (*Proc. natn. Acad. Sci. U.S.A.*, **17**, 1342–1346; 1974) have sequenced the terminal portion of the rRNA from several bacteria and found that certain oligonucleotides near to the 3′-OH are complementary to GGAGGU—a sequence common to many of the mRNA ribosome binding sites that have so far been examined. Perhaps such a structure selects the correct site of mRNA and positions it on the ribosome. It is conceivable that the small RNA species described above has a similar function in eukaryotes.

The new RNA species not only has initiation factor activity but can also discriminate between different mRNAs. This means that a change in the specificity of initiation can be effected either by a protein (IF$_3$) factor or by an RNA species, and adds a further dimension to experiments in which changes in the specificity of initiation factors are being examined, for example, at different stages in development (Sierra, Meier and Ochoa, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2693–2697; 1974). The presence of small RNA molecules in initiation factor preparations from uninfected cells may also suggest a function for those ribosome-associated RNA molecules that are found during viral infection but apparently do not function as mRNAs. For example during reovirus and adenovirus infections, small viral RNA molecules have been detected in excess of those required to code for viral proteins—these RNAs could alter the specificity of translation on host cell ribosomes, either by shutting off host cell synthesis or preferentially favouring viral protein synthesis.

Double-stranded RNA also has a profound effect on the initiation of protein synthesis. This was first described by Ehrenfeld and Hunt (*Proc. natn. Acad. Sci. U.S.A.*, **68**, 1075–1078; 1971) who were searching for the inhibitory agent responsible for shutting off host protein synthesis in poliovirus-infected cells. The effect on initiation was detected in rabbit reticulocyte lysates and was remarkable because of the small amounts (about 0.1 ng ml^{-1}) of ds RNA required. Celma and Ehrenfeld (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 2440–2444; 1974) have now reassessed the possible role of ds RNA in the shut-off phenomenon by studying the initiation of protein synthesis in cell-free extracts of uninfected and poliovirus-infected HeLa cells. Host and viral protein synthesis are equally sensitive to ds RNA and much higher concentrations (about 5 μg ml^{-1}) of RNA are required to inhibit synthesis in the HeLa cell-free system. Since such concentrations of inhibitor are not present until late in poliovirus infection Celma and Erhenfeld suggest that ds RNA does not act directly to inhibit host cell protein synthesis. Such a conclusion is supported by experiments reported in the same issue of *Proc. natn. Acad. Sci. U.S.A.* (**71**, 2549–2553; 1974) by Thach and colleagues, who have determined by electron microscopy the structure of the purified replicative intermediate of encephalomyocarditis virus (a picornavirus closely related to polio). These authors conclude that before deproteinisation the replicating RNA contains very little ds RNA, thus the inhibitory agent detected in polio-infected cells may be an artefact caused by extraction of the viral RNA.

But three further considerations should be weighed in favour of ds RNA as the inhibitory agent before it is completely rejected. First, cell-free extracts from HeLa cells are never very active compared with whole cells; second, disrupted cells may contain an RNase III type activity which destroys much of the added ds RNA but is without such activity *in vivo* and finally even though full length ds RNA may not be present in infected cells presumably the area immediately surrounding the polymerase molecule is in a double stranded form; the effect of ds RNA in

Shades of meaning



Male quagga in Naturhistorisches Museum, Mainz on left; Burchell's zebra (type of *E. quagga paucistratus*) on right.

THE quagga, *Equus quagga quagga*, no longer roams the Cape Colony having succumbed to the hunter's gun in the nineteenth century. The last quagga died in the Amsterdam Zoo in 1883, but it is thought that this one had outlived by several years the wild quaggas in South Africa.

The striping and coloration of this close relative of the modern zebra have long been contentious subjects. There has also been controversy about whether the quagga is a true subspecies or just a geographical variant of Burchell's zebra, *E. q. burchelli*. In an attempt to sort out the confusion, Rau of the South African Museum in Cape Town, has scoured Europe for quagga remains and he now presents his revised list of what he considers true quagga material (*Ann. S. Afr. Mus.*, **65**, 41–87; 1974).

In the past, the quagga has been described variously as having black or dark stripes on a brown or fawn base with white or whitish legs and ventral surface; or being dark brown, chestnut or fawn coloured with white or whitish stripes on the antero-dorsal area of the animal. Authors writing this century have favoured a light striped animal.

After studying preserved quaggas in 27 European museums Rau concludes that there is no sharp distinction between the quagga, *E. quagga quagga* and *E. q. burchelli*. It just seems to represent, with many variations, the extreme southern limit of the trend seen in the plains zebra group: that is, in the south, the white darkens towards brown and the black lightens to a brown. This process gradually culminates in the disappearance of the stripes.

reticulocyte lysates, which lack RNase III, is so potent that this small region of poliovirus ds RNA might be sufficient to produce the inhibitory effects seen *in vivo*.

The mechanism of action of ds RNA in reticulocyte lysates is the subject of another recent paper in *Proc. natn. Acad. Sci. U.S.A.* Benzard and London (**71**, 2863–2866; 1974) have shown that the inhibitory effect can be prevented or reversed by addition of the initiation factor M_3. Preparations of initiation factors M_1 and M_2 (which are responsible for the binding of initiator tRNA) and ribosome dissociation factor are ineffective. These observations are in agreement with similar experiments by Kaempfer and Kaufman (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 1222–1226; 1973) who showed that ds RNA inactivates reticulocyte initiation factor IF_3, but both reports conflict with those of Darnbrough, Hunt and Jackson (*Biochem. biophys. Res. Commun.*, **48**, 1556–1564; 1972) who found that ds RNA inhibits the formation of the complex between 40S ribosomal subunits and initiator tRNA. At present it is difficult to resolve this apparent contradiction. But it seems that very small amounts of ds RNA inhibit initiation by sequestering a catalytic site which normally recognises some structural feature in another RNA molecule. Such a structure could be present in mRNA, rRNA, initiator tRNA or perhaps even the small RNA molecule discussed earlier.

Archaeopteryx flies again

from Barry Cox

Archaeopteryx is probably the best example of an evolutionary intermediate between two major groups of animal. It is normally accepted that it is not far removed from the arboreal reptilian stock from which birds evolved. Though its skeleton is not greatly modified for flight, the elongated feathers of *Archaeopteryx* strongly suggest that flight of some kind had already evolved.

Ostrom (*Q. Rev. Biol.*, **49**, 27; 1974) has now questioned the idea that bird flight evolved in an arboreal environment. He starts from the observation that *Archaeopteryx* and the smaller cursorial dinosaurs such as *Ornitholestes* show so many characteristics in common that it would be surprising if their habits of life were totally different. He also states that the terminal phalanges of the foot of *Archaeopteryx* are relatively straight and robust, like those of cursorial birds but unlike those of perching birds, which are strongly curved.

It has long been noted that, surprisingly, the manus of *Archaeopteryx* has three long, unfused clawed fingers, and these have been interpreted as adaptations to climbing in trees. Ostrom shows that the hand, and the whole pectoral skeleton, of *Archaeopteryx* are very similar to those of such dinosaurs as *Ornitholestes*, and that the claws were long, curved and pointed—more suitable, he thinks, to a prey-grasping function that to climbing. He also notes that neither the pectoral skeleton nor the long many-feathered tail of *Archaeopteryx* suggests that it could have been an active flier.

Ostrom's view is that *Archaeopteryx* was a bipedal fast-running form which lived on "relatively small animals, most probably large insects, and perhaps small lizards and mammals". He believes that feathers evolved to aid in thermal insulation, and that their elaboration on the forelimb was originally to increase its efficiency as a prey-catching appendage, the two arms "becoming a trapping device or net with which to corral or surround small prey so that they could be more easily grasped in the mouth or hand". True flight, Ostrom believes, evolved from flapping, leaping attacks on such prey.

Long-established theories are often found to be based on deductions which are today either discarded or less certain, and it is usually worthwhile to attempt a fundamental reappraisal. Ostram's work seems certain to provoke further discussion and criticism—both of the orthodox theory and of his own views. Walker (*Nature*, **237**, 257; 1972) has already suggested that birds are more closely related to crocodiles than to theropod dinosaurs, but both Gingerich (*Nature*, **243**, 70; 1973) and Bakker and Galton (*Nature*, **248**, 168; 1974) have supported the link with dinosaurs—the latter even to the extent of including birds in a new class Dinosauria.

Some aspects of Ostrom's theories seem somewhat unlikely, at least at first glance. It is always heartening to the palaeontologist to be able to see similar adaptations in use today, but there are none such for *Archaeopteryx's* supposed insect-snaring spread of feathers. These would also have been of little use (and possibly a hindrance) in catching the small lizards and mammals that Ostrom proposes as alternative prey. His suggestion that the long, feathered tail was a braking mechanism seems equally unconvincing. Ostrom may well be right in suggesting that the ancestors of *Archaeopteryx* evolved bipedality as ground-running forms, like some modern lizards (in which bipedal and quadrupedal locomotion are not fundamentally different), and that they only evolved the extensive spread of feathers as a gliding adaptation after they had become arboreal forms. Nevertheless, the adoption of a dramatically different method of locomotion (flight) might well have followed from colonisation of a very different habitat, such as trees. The imperfection of *Archaeopteryx* as an arboreal gliding form and its retention of a grasping hand might then merely indicate a relatively recent adoption of this way of life, rather than disproving it.

Other interested parties are girding their loins for the fray, and it will be interesting to see whether *Archaeopteryx* ends up with its feet on the ground or its head in the air.

Agricultural waste to supplement oil

from D. A. H. Taylor

In 1949 Dupont opened a plant at Niagara for the production of nylon intermediates from furfural. This has long been closed down, as it could not compete with petrochemical routes, but it may now be time to reconsider the position of furfural in the light of the present oil situation.

Furfural is obtained by acidic hydrolysis of pentosans, contained in agricultural waste. Almost any plant material may be used, including straw and sawdust, with an average yield of 5–10%. Present sources include oat hulls, maize cobs, and bagasse, which is the residue after extracting sucrose from sugar cane. The potential supplies are large: it has been calculated that 2 million tons of furfural could be produced per annum in the United States from maize cobs alone (Dunlop and Peters, *The Furans*, 283, Reinhold, New York; 1953); the potential yield from sugar cane is roughly equal to that of sucrose, currently about 50 million tons yr^{-1}. This may be compared with a world production in 1970 of approximately 40 million tons of primary petrochemicals (Imhausen, *Chem. Ind.*, 1559; 1970.)

It is possible to make practically any petrochemicals one wishes from furfural, at a price. As the sources of furfural are usually of slight alternative value, the main expenses in production are plant construction, operating costs and handling and collection charges for the raw material. Its present uses are mainly as a solvent and in the synthesis of resins used to manufacture grinding wheels and brake linings. A major use is the production of resin for impregnating the glass fibre mats wrapped around steel pipelines to prevent corrosion, and currently the demand in connection with the construction of Arctic pipelines has raised the price of furfural delivered in Europe from less than £200 to more than £650 a ton. By comparison, the current price of naphtha feedstock, used for the production of petrochemicals, is of the order of £60 per ton. At this level, oil prices have still far to go before furfural could compete as a fuel for heating or power plant use. Furfural itself does not seem to have attractive properties for use as a fuel for internal combustion engines, but chemical derivatives are more promising.

Furfural derivatives usually require hydrogenation to be useful chemically which is unfortunate because hydrogen is rich in energy and hence expensive. The Dupont nylon process first decarbonylated furfural to furan by catalytic treatment, and then hydrogenated the furan to give tetrahydrofuran. This was then cleaved with hydrogen chloride to form 1, 4-dichlorobutane, which with sodium cyanide gave adiponitrile, a conventional intermediate for nylon 6/6.

Tetrahydrofuran is a valuable intermediate in other ways; one fifth of German wartime butadiene was produced by vapour phase dehydrogenation of tetrahydrofuran over a phosphate catalyst (Dunlop and Peters, *The Furans*, page 730). Although no longer used, this process would become viable again if alternative supplies of butadiene became more expensive, and gives access to a wide range of products, including the synthetic rubbers. Tetrahydrofuran has also potential as a fuel for internal combustion engines. Although its properties in this respect have not been investigated in detail it seems promising either alone or together with hydrocarbons, with which it is readily miscible.

The tetrahydrofuran process involves the loss of one carbon atom from the furfural molecule, and an alternative route which avoids this is to hydrogenate furfural to tetrahydrofurfuryl alcohol. This on heating in the gas phase gives 2, 3-dihydropyran, which may be hydrogenated to tetrahydropyran. This is also a potential fuel and chemical intermediate; analogously to tetrahydrofuran it can be converted to 1, 3-pentadiene, or to pimelonitrile and hence to nylons of the 7/7 series. Extensive development work has been done on these and other possible routes for utilisation of furfural in the chemical industry.

Hydrogenation of butadiene or pentadiene will give the saturated butane or pentane, which are in the main stream of petrochemical practice. Butane is currently used to manufacture motor fuel.

Thus increased furfural production may help to solve at least some of the present and future problems produced by the increased price and declining supply of crude oil.

Protein in two environments

from R. Colin Hughes

THE classical concept of amphipathic membrane proteins defined by Winzler, Morawiecki, Singer and their colleagues is a polypeptide containing separated domains of peptide sequences of polar or non-polar character. This seems to occur in the major erythrocyte membrane glycoprotein, a molecule in which oligosaccharide units are attached exclusively to the amino terminal half of the polypeptide. The carboxyl terminal half of the chain contains an amino acid sequence which is unusually hydrophobic and seems to be embedded in the lipid bilayer of the membrane. As Singer has pointed out, however, an amphipathic protein may consist of a linear polypeptide of more mixed character that only when folded produces the two distinct segments, one highly polar and the other highly hydrophobic, required of an integral membrane protein. Sequence analyses of more membrane proteins are obviously of great interest, and information of their three-dimensional structure cannot be too long delayed.

In the meantime, however, the field is open for speculation. In the June issue of the *Proceedings of the National Academy of Sciences* (**71**, 2396–2400; 1974) Inouye produces a particularly ingenious model for one membrane protein—the lipoprotein of the outer membrane of the *Escherichia coli* envelope.

The bacterial envelope consists of two distinct membranes: the inner or cytoplasmic membrane and the outer membrane, which are separated by a thin layer of peptidoglycan. The outer membrane contains a large amount of lipopolysaccharide and a unique lipoprotein, thoroughly characterised by Braun and his colleagues. The lipoprotein just penetrates the lipid bilayer of the outer membrane to peep out both at the bacterial external surface and at the inner surface towards the peptidoglycan, to which the lipoprotein is in part covalently linked. The sequence data of Braun show that the lipoprotein consists of 58 amino acid residues. An unusual amino acid, glycerylcysteine [S-(propane-2′, 3′-diol) 3-thio-2 amino-propanic acid] is N-terminal and linkage to the peptidoglycan involves the C-terminal lysine residue, which is linked to the carboxyl group of meso-diamino pimelic acid.

The lipoprotein has a high α-helical content and Inouye puts forward two possible arrangements in which the α helix is twisted to allow the hydrophobic amino acid residues to face in one direction whereas the hydrophilic residues face oppositely. These arrangements are of course compatible with an integral membrane protein, provided that the twisted helices are aggregated to form a superstructure in which the hydrophilic faces are in contact and the outer surface of the structure is hydrophobic. This is satisfied either by a cylindrical arrangement of six helices or by six α-helical coils arranged in a superhelix or a coiled coil, with the hydrophobic residues of each α helix pointing outwards. The height of the superhelical assembly will be about 76 Å, sufficient to penetrate through the 75 Å thick outer membrane with hydrophobic interaction between the surface of the assembly and the lipid bilayer of the outer membrane. The lipoprotein molecules may be stabilised further in the bilayer through lipid–lipid interactions involving the three fatty acids attached to the N-terminal residue (two by ester linkage and one by amide linkage). Since one or more of the individual α-helical polypeptides of the assembly may be attached covalently to the underlying peptidoglycan, the lipoprotein assembly is constrained and free diffusional motion within the outer membrane presumably is not allowed.

The function of the bacterial lipoprotein is unknown. Inouye points out an intriguing aspect of his model, however, in that the superstructure of assembled helices spanning the membrane could provide hydrophilic pores through the outer bacterial membrane. Two general classes of molecular mechanisms for protein-mediated transport involving pores may be postulated. A penetrating membrane protein could be itself porous. Alternatively, the pores could be formed by an assembly of protein molecules: the proteins are porogenic rather than porous. In the case of the bacterial lipoprotein, Inouye calculates a hydrophilic pore size of from 12.5 Å to 35.8 Å depending on an assembly of six or twelve α helices. The number of lipoprotein molecules per bacterium is about 7.5×10^5, and accordingly the calculated total number of channels per cell varies from 1.25×10^5 to 0.63×10^5. That is, about 35% to 45% of the total bacterial cell surface is occupied by the assemblies. These hydrophilic channels could accommodate the passive diffusion through the outer bacterial membrane of almost all materials required for growth, provided of course that diffusion of these substances is also allowed through the other barriers of the peptidoglycan layer and the cytoplasmic membrane

articles

Search for pulsed X rays from 3U0833-45 (Vela pulsar)

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A search for pulsed X rays from 3U0833-45 has been carried out. The Uhuru error box for this source (~8′ × 24′) contains the Vela pulsar. We obtain an upper limit of ~1/3 the intensity of 3U0833-45 for periodic pulsing with the 89-ms period of the Vela pulsar. Other evidence for X-ray emission from the Vela pulsar is reviewed.

SOME 100 radio pulsars have been discovered. The spin periods range from 33 ms (NP0532) to 3.75 s (NP0525). Only two of these pulsars have been reported to emit X radiation: the Crab pulsar (NP0532) and the Vela pulsar (PSR0833-45). In the case of NP0532, the pulsed X-ray emission is rather intense, making it the 25th brightest X-ray source in the sky. Its high energy emission covers the range from 1 keV to greater than 1 GeV (refs 1–3).

The Vela pulsar is the next most likely emitter of X rays because it has the next highest spin frequency, ~89 ms. There have been various reports of X-ray emission, and lack of emission, from it. In the 2 to 10 keV range, a source (3U0833-45) has been detected in the vicinity of the Vela pulsar by Uhuru[4]. The error box for this source is ~8′ × 24′ and contains the Vela pulsar; however, the time resolution of Uhuru is insufficient to reveal a possible 89 ms pulsing. The diffuse Vela supernova remnant has not been detected in the 2 to 10 keV energy range.

A marginally significant detection of X-ray pulsing from the Vela pulsar in the energy range 23 to 80 keV has been reported[5], but with a period 155 ms different from the radio period. In the same energy range the MIT balloon group found no pulsing (2σ confidence) at the same level of intensity[6]. In soft X rays, 0.5 to 1.5 keV, the AS&E group found pulsing with the same period as in the radio and with an intensity of ~2% of the total X-ray intensity of the Vela supernova remnant[7]. The same group also reported a non-pulsed component about twice as intense as the pulsed component[7]. These latter results are inconsistent with a recent pulsing limit set by the CalTech group[8] (<0.3% of the intensity of the Vela supernova remnant) and the detection by the Copernicus satellite of only a much weaker source within 6′ of the Vela pulsar[9]. The time resolution of the Copernicus X-ray instrumentation during the Vela observation was inadequate to detect 89 ms pulsing. The experimental results are summarised in Fig. 1.

Our data

The present experiment, which features 1 ms time resolution, was directed toward a search for periodic (89 ms) pulsed X-ray emission from 3U0833-45 in the energy range 1.5 to 10 keV. An earlier search in the same energy range[10] yielded an upper limit substantially above the intensity of 3U0833-45. The present observations were made from an Aerobee 170 sounding rocket launched from Woomera (Australia) at 1800h, November 9, 1973 (UT). The payload instrumentation consisted of a bank of 6 beryllium window proportional counters, sensitive in the energy range 1.5 to 10 keV with 900 cm² total open area. During the final 100 s of the flight, the view axis of the detectors was pointed in an intermediate direction between Vela X-1 (3U0900-40) and the Vela pulsar. Only 70 s of the Vela data were recovered because of a single 30 s loss of the telemetry signal. The instrumentation and the results of the observations of Cir X-1 (observed earlier in the flight) and Vela X-1 have been presented previously[11].

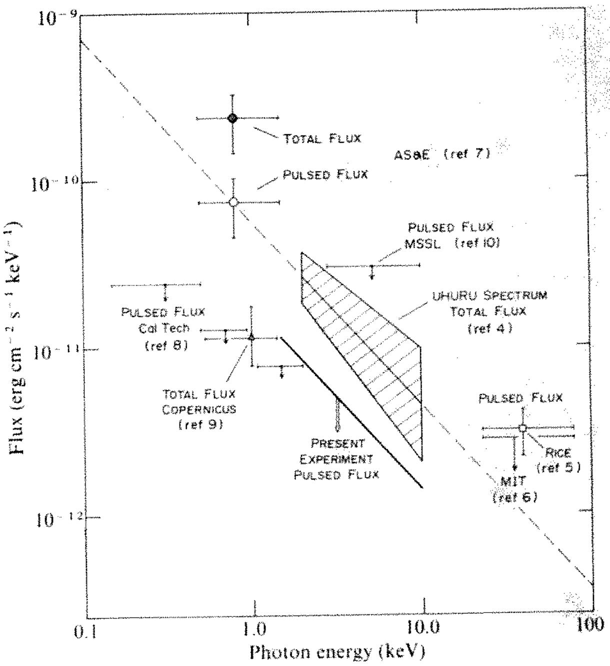

Fig. 1 Summary of X-ray data for the Vela pulsar. The pulsing limits from the present experiment (1.5 to 10 keV) are derived under the assumption of an energy spectrum with a spectral index $\alpha = 1.1$ (same as for 3U0833-45, ref. 4). For different assumed spectra, the limits set by the heavy line in the figure would rotate about a fixed point near 3 keV (5×10^{-12} erg cm^{-2} s^{-1} keV^{-1}) where the detectors are most sensitive.

The observations in the Vela region yielded about 6,500 signal counts above a background of ~38,000 counts, due mostly to the isotropic X-ray flux. We estimate that 3U0833-45 contributes about 600 of the 6,500 signal counts. We arrive at this estimate by folding the spectrum of 3U0833-45 (ref. 4: $I(E) = 5.6 \times 10^{-11} E^{-1.1}$ erg cm^{-2} s^{-1} keV^{-1}) with the response function of our detectors. The calculation indicates a total source counting rate for the present experiment of 9 counts s^{-1}. The data were folded modulo the apparent period of PSR0833-45 at the time and place of the rocket launch, $P = 0.0892230$ s

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(private communication from P. Reichley). The resultant histogram (Fig. 2) shows no obvious or statistically significant features. The hatched peak superimposed on the data represents the expected appearance of the light curve if 3U0833-45 were 100% pulsed and had a light curve identical with the radio pulse profile of PSR0833-45 at 2.4 GHz (single pulse ~4 ms wide[12]).

Implications for models

So most of the signal from the Uhuru source 3U0833-45 is either pulsed with a wide duty cycle ($\gtrsim 30\%$) or is a steady non-pulsing flux. A steady X-ray flux could be synchrotron radiation from a nebular source surrounding the pulsar although there have been no reports of a compact steady non-thermal radio source[13]. The observed emission cannot be blackbody radiation from the hot neutron star surface since the required temperature, $\sim 10 \times 10^6$ K, is too large for the current age ($\sim 10^4$ yr, ref. 14) of the pulsar. Also, the X-ray flux at the Earth would be almost two orders of magnitude larger than observed, even for a minimum neutron star radius of ~5 km and a distance of ~1 kpc, which is twice that given in ref. 15.

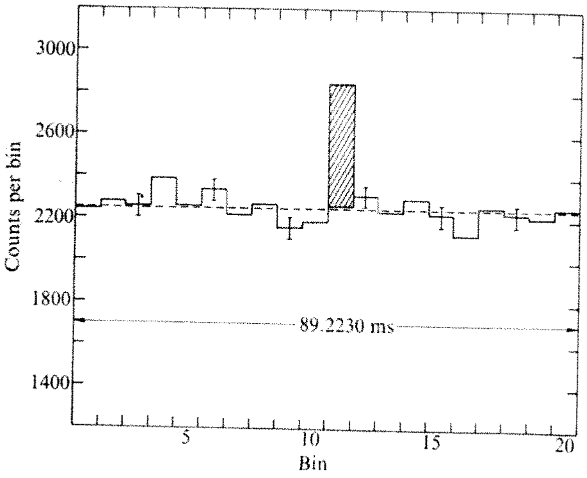

Fig. 2 X-ray data from the Vela region superimposed modulo the apparent period of PSR0833-45. The time reference for zero phase, (start of bin 1) is the launch time: 18 h 00 min 00 s, November 9, 1973 (UT). About 99% of the counts are from the isotropic X-ray background and 3U0900-40. The hatched peak represents the expected light curve if 3U0833-45 were 100% pulsed with a duty cycle similar to that of the radio pulsar. The phase of the expected peak is arbitrary since we were unable to obtain the phase of the radio pulse.

The limit (3σ) for the time averaged pulsed X-ray emission is 3×10^{-11} erg cm^{-2} s^{-1} (1.5 to 10 keV) or 5×10^{-12} erg cm^{-2} s^{-1} keV^{-1} at 3 keV for an assumed $E^{-1.1}$ energy spectrum and pulse profile similar to that of the radio pulse. In this context, we note that the X-ray and radio pulse shapes for the Crab pulsar are similar[1,16]. If the width of the X-ray pulses from PSR0833-45 is as broad as 12 ms, then the upper limit to pulsed emission which we can set is about 5×10^{-11} erg cm^{-2} s^{-1}. The time averaged X-ray luminosity then becomes

$$L_{\mathrm{Vela}}\ (1.5\ \text{to}\ 10\ \text{keV}) \leqslant 8 \times 10^{32}\ \text{erg s}^{-1}$$

for a distance of 500 pc and a 4 ms pulse produced by a broad fan beam. This compares with the X-ray luminosity of the Crab pulsar, for a distance of 2 kpc (refs 1, 17):

$$L_{\mathrm{NP0532}}\ (1.5\ \text{to}\ 10\ \text{keV}) = 9 \times 10^{35}\ \text{erg s}^{-1}$$

The Crab pulsar therefore radiates at least 1,000 times the power of the Vela pulsar in the 1.5 to 10 keV range. This ratio is still greater than 150 if the Vela pulsar is as far as 1 kpc and the Crab is as close as 1.5 kpc. If the X-ray luminosity of pulsars has a power law dependence on the spin period, P, ($L_x \propto P^{-n}$), then these ratios yield an index n which is probably greater than seven and conservatively greater than five. The value $n = 4$ is predicted for the total luminosity by the oblique rotator or unipolar inductor models[18-20]. If one further assumes that a fixed fraction of the rotational energy loss is emitted in the form of pulsed X-rays (ref. 21), then the present result is incompatible with these models. The luminosity dependence on spin period predicted by the "corpuscular model" ($L \propto P^{-7}$, ref. 22) and that predicted for incoherent synchrotron radiation close to the speed-of-light circle ($L \propto P^{-10}$, ref. 23) seem to be consistent with the present X-ray observations. The calculated X-ray luminosity for incoherent radiation from the "electron polar zones" ($L \propto P^{-17/4}$, ref. 24) is inconsistent with the present result both in its dependence on spin period and in its absolute value.

A similar comparison at optical wavelengths has been made previously. The time averaged apparent magnitude of NP0532 is $m_v = 16.5$. No detections of PSR0833-45 have been made down to limiting magnitudes $m_v \sim 24$ (refs 25, 26). This leads to a conservative upper limit to the optical luminosity ratio[25]:

$$L_{\mathrm{Vela}}/L_{\mathrm{NP0532}} < 2 \times 10^{-4}$$

which is even smaller than in the X-ray region. The index n for the optical case is then greater than ~8.5, but this holds only if there is no severe optical extinction for the Vela pulsar.

Further X-ray observations of 3U0833-45 are required to provide an improved position to confirm the identification with the pulsar and to search for lower levels of pulsed X-ray emission.

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Implications of heat flow for metallogenesis in the Bauer Deep

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The Bauer Deep, in the south-eastern equatorial Pacific, is the only deep ocean basin so far known to be characterised by metalliferous sediments throughout the depositional column. The basin was formed when the eastern Pacific spreading centre jumped from the now fossil Galapagos Rise to the newly formed East Pacific Rise.

The distinctly bimodal distribution of heat flow in the Bauer Deep indicates that precipitation from hydrothermal fluids emanating from the oceanic crust may be the mechanism for metallogenesis in the Bauer Deep. The hydrothermal circulation may have been initiated by the emplacement, approximately ~ 6 Myr BP, *of a heat source associated with the ridge jump into that vicinity.*

THE existence of metalliferous sediments on the East Pacific Rise and other mid-ocean ridges has been known for some time[1-3]. Such deposits are a characteristic of basal sediments from Deep Sea Drilling cores throughout the ocean basins (see refs 4–6). The Bauer Deep in the south-eastern Pacific, is the only deep-ocean basin known at this time in which metalliferous sediments have been reported throughout the depositional column. Sayles and Bischoff[7] reported concentrations of Fe and Mn of up to 18% and 6.5%, respectively, and low concentrations of Al and Ti in sediments containing little or no $CaCO_3$. They also reported anomalously high concentrations of Cu, Ni, and Zn.

Extensive mapping of the regional extent of the ferriginous sediments has been an integral part of the IDOE Nazca Plate project (G. R. Heath *et al.*, unpublished). Cores from JOIDES, leg 34, site 319 (Fig. 1) have recently shown more than 100 m of metalliferous sediment in the Bauer Deep[8], with large concentrations of metalliferous components throughout the entire section. Cores from leg 34, holes 320 and 321 (Fig. 1) also revealed basal segments rich in heavy metals, although the upper sections of each were relatively free of anomalous metal concentrations.

Corliss[9] argued that metallic sediments from the mid-ocean ridge crest were precipitated from hydrothermal solutions percolating upwards from the basaltic oceanic crust. He argued that metals are mobilised by dissolution in seawater circulating in the crestal provinces of ridges, Williams *et al.*[10], Lister[11], and Sclater *et al.*[12] have presented extensive evidence, based upon a bimodal, oscillatory heat-flow pattern, for massive hydrothermal circulation in the crestal province of mid-ocean ridges. The seafloor spreading process itself thus provides the heat source, volcanism, faulting, and fracturing necessary to drive hydrothermal circulation in this environment.

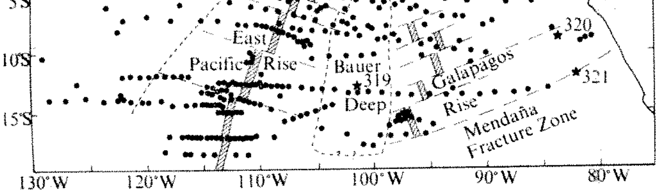

Fig. 1 Physiography of the south-eastern equatorial Pacific Ocean. Solid circles, locations of heat-flow stations plotted in Fig. 2 (sources from refs 20, 27–31, and R. N. Anderson *et al.*, unpublished); hatching, active and inactive spreading centres; light dashed lines, fracture zones; stars, JOIDES, Leg 34, hole locations[8].

Sediments from the Bauer Deep are chemically similar to sediments from the East Pacific Rise in that both have high transition metal: aluminium ratios[13-15], with sulphur, uranium, and O_2 isotopic compositions consistent with formation from seawater[13,14], strontium isotopic compositions suggesting equilibrium with seawater[14,15], rare-earth patterns like that of seawater[9,13], and lead isotopic compositions which are similar to mid-ocean ridge tholeiites[15]. The mineralogies of the two areas are, however, quite distinct —the ridge crestal metals are found as oxides and the Bauer Deep metals are found as silicates[7]—and the mode of formation is by no means settled.

The origin of these metalliferous sediments and, in general, iron–manganese deposits throughout the oceans is still an unsolved problem. Bonatti *et al.*[16] suggested four different mechanisms of origin. First, hydrogenous, that is, slow precipitation from seawater in oxidised environments. These deposits are found as microparticles dispersed in sediments, nodules and concretions on abyssal plains, and solid pavements on topographic highs. Second, hydrothermal, that is, precipitation from hydrothermal solutions in areas of volcanism and/or high heat flow. These deposits are found along active oceanic ridges and rifts and are associated with submarine volcanism. Third, halmyrolytic, that is, manganese supplied by submarine weathering of basaltic debris. Fourth, diagenetic, that is, precipitation resulting from diagenetic remobilisation of manganese in the sediment column.

There are at least three major hypotheses for the occurrence of metallogenesis in the Bauer Deep. Metals may be precipitated from hydrothermal solutions actively circulating there today[15]. Alternatively, they may be hydrogenous and may have nothing whatsoever to do with hydrothermal circulation in the basin. Excessively slow sedimentation rates could produce the large concentrations of metallic components; or some metals may be released into the water column at mid-ocean ridges by hydrothermal circulation and others may be hydrogenously deposited in the Bauer Deep[13]. Little geophysical evidence has so far been brought to bear on this problem.

We present a compilation of heat-flow measurements in the area and draw upon the anomalous nature of the thermal structure of this basin to propose that extensive emanation of hydrothermal fluids[15] can account for both the observed metalliferous sediments and the heat-flow distribution in the Bauer Deep. A qualitative model is presented, associating both with the emplacement of a new heat source into old oceanic crust during the jumping of the major north–south seafloor spreading centre in the eastern Pacific from the Galapagos Rise to the East Pacific Rise.

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Physiography

The Bauer Deep is bounded on the west by the East Pacific Rise, on the north by the Galapagos Spreading Centre (both are active seafloor spreading mid-ocean ridges), on the east by the Galapagos Rise, a fossil spreading centre, and extends to the south to at least the Mendaña fracture zone at 16° S on the Nazca Plate (Fig. 1). The basin was formed by the westward jumping, about 6 Myr ago, of the spreading centre from the Galapagos Rise[17,18], 800 km to the west to form the East Pacific Rise in crust which was at least 10 Myr old at the time of the jump[17,19].

The Bauer Deep has a marked topographic step bordering the basin on the west, and a relatively smooth slope up to the Galapagos Rise on the east (Fig. 2). The elevation offset across this western step indicates an age discontinuity from crust which is about 6 Myr old, and which was generated at the crest of the East Pacific Rise to the crust of the Bauer Deep which is about 10–16 Myr old and which was generated at the Galapagos Rise.

The western boundary of the basin is also marked by extremely rough topography. These ridges are probably not primary topographic features emplaced at the spreading axis, but are either uplifted basement[8] or extrusive ridges emplaced at the time of the ridge jump. Basement at site 319 (Fig. 1) is extremely fresh compared with other drilled basalts recovered just below basal sediments of the same age (~ 15 Myr) and the composition of basalts is more alkaline than tholeiites from the ridge crest[8]. Therefore, the basement could be 'off-ridge extrusions' younger than the basal sediments[8].

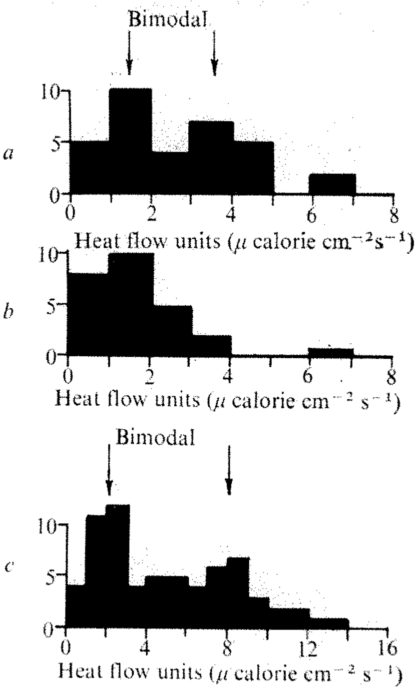

Fig. 3 Histogram of heat-flow values in *a*, the Bauer Deep—$n=33$—(crustal ages 10–16 Myr BP, from Fig. 2); b, the eastern Pacific—$n=26$—(ages 10–16 Myr BP, from Fig. 6 of ref. 20); *c*, the Galapagos Spreading Centre—$n=67$–(0–1 Myr BP, from ref. 10).

Heat flow distribution

Our heat-flow compilation is part of an extensive discussion of the heat-flow distribution on the Nazca Plate (R. N. Anderson *et al.*, unpublished). A heat flow against age plot for the eastern Pacific shows that the Bauer Deep is characterised by high, but variable heat flow (Fig. 2). The variability of heat flow commonly decreases as the average heat flux decreases with age away from mid-ocean ridges[20]. The mean and standard deviation of heat-flow values from the Bauer Deep are twice as large as values for oceanic crust of the same age in other ocean basins (see ref. 20). The observed mean heat flow departs significantly from the heat flux predicted by an empirical relationship between heat flow and age compiled for the entire eastern Pacific[20] in one area only—the Bauer Deep (Fig. 2). High heat flow and large scatter in values are evident around the crests of the East Pacific Rise and the Galapagos Rise, as well as in the Bauer Deep. This is a characteristic of mid-ocean ridge heat flow patterns in general (see ref. 10). For crust 10–16 Myr old (the age of the Bauer Deep) the empirical heat flow against age relationship[20] was determined from 26 heat flow measurements in areas of the eastern Pacific away from ridge jumps and fossil ridges. These 26 measurements do not show the bimodal heat flow pattern characterised by the Bauer Deep values (Fig. 3). Rather, they show a normal distribution about their mean, of 1.4 μcalorie cm^{-2} s^{-1} (~60 mW m^{-2}), (Fig. 3). A comparison of crust 10–16 Myr old, east and west of the Galapagos Rise (Fig. 2), again shows the anomalous nature of the thermal structure of the Bauer Deep. Such variability is characteristic of conductive heat flow measurements in areas dominated by convective heat transfer which is associated with hydrothermal circulation[10–12]. Heat flow values from the Bauer Deep (Fig. 3) show a distinct bimodal distribution characteristic of hydrothermal circulation areas such as the Galapagos Spreading Centre (Fig. 3). The heat-flow pattern at the crest of that mid-ocean ridge shows a 5 km wavelength oscillation of heat flow away from the ridge. Variations from 0.1 μcalorie cm^{-2} s^{-1} (4.1 mW m^{-2}) to >33 μcalorie cm^{-2} s^{-1} (>790 mW m^{-2}) were measured over a 4 km distance. This variability, the systematic oscillation, and observed water temperature anomalies which indicate hot spring activity led Williams *et al.*[10] to comment that there was conclusive evidence for widespread circulation of seawater into the oceanic crust on the Galapagos Spreading Centre.

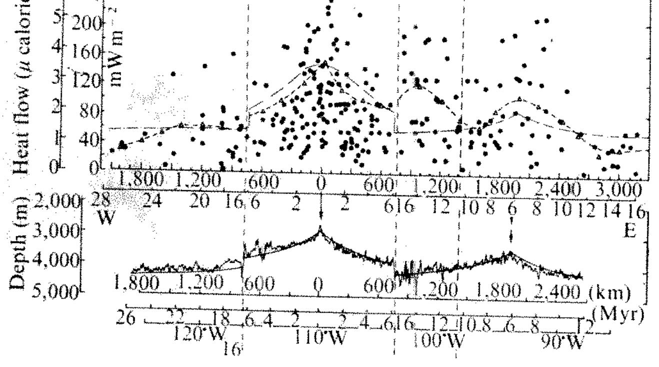

Fig. 2 Top, heat flow plotted against age for the south-eastern equatorial Pacific. Only those measurements shown in Fig. 1 are used for this compilation. Bottom, composite bathymetric profile (from Anderson and Sclater[18]) showing representative topographic features in the area. Solid circles, heat-flow values; open triangles, means for 400 km overlapping intervals; solid curves, empirical elevation against age (from ref. 25); — · —, empirical heat flow against age (from ref. 20); dashed line, averaged observed heat flow for the south-east Pacific.

In addition, thermal conductivity (K) measurements in the Bauer Deep made both on recovered cores[21] and *in situ*[22] produced some values 100% greater than those measured in 'typical' deep-ocean sediments. Values of 3.9 and 3.7 mcalorie cm^{-1} s^{-1} °C^{-1} (1.6 and 1.55 W mK^{-1})

were measured at 14°37.2′S, 104°33.2′W, and 14°53.3′S, 105°15.8′W (R. N. Anderson *et al.*, unpublished). The banding of layers of high conductivity was measured in cores from the Galapagos Rise (A. J. Halunen, jun., unpublished). Thus, the average heat flow in the Bauer depression (2.65 μcalorie cm^{-2} s^{-1} (~100 mW m^{-2}) for 33 measurements) may be biased low because most measurements have been determined by assuming conductivities from nearby locales rather than from measurement of sediments collected at the site. The existence of the newly discovered high conductivities in the metalliferous region implies to us that many assumed conductivity values may be as much as 25% too low.

Metallogenesis model

Spooner and Fyfe[24] have presented a model for subseafloor metamorphism which involves the circulation of seawater through oceanic crust at mid-ocean ridges, chemical exchange with basalt during hydration of the oceanic crust, and subsequent upwelling of hot, hydrothermal solution into the sediment column. They argue that inorganic precipitation from the hot brines can account for heavy metal enrichment found in ophiolitic cherts.

As already mentioned, Bauer Deep sediments have sulphur, uranium, oxygen, and strontium isotopic compositions and rare-earth element patterns consistent with formation from seawater[13-15], and lead isotopic compositions similar to those of mid-ocean ridge tholeiites[9]. The latter led Dasch *et al.*[15] to conclude that the iron-rich sediments from the Bauer Deep originate as precipitates from hydrothermal solutions injected into sea water. Bischoff and Sayles[25] point out, however, that the *p*H and major ion concentrations of the pore fluids from the core examined by Dasch *et al.*[15] do not differ significantly from the ion concentrations in the overlying bottom water. Sayles and Bischoff[7] therefore concluded that "at this point, it is not possible to evaluate the role of 'volcanic emanations' in supplying the major components of Bauer Deep sediments". We wish to add the previously unrecognised geophysical constraint of anomalously high and variable heat flow to the accumulating body of data, mostly geochemical, which will ultimately lead to the determination of the mode of origin of metalliferous sediments in the Bauer Deep.

A model can be suggested which can account for both the anomalous thermal structure and the metalliferous deposition in the Bauer Deep. The important distinction to be made is that hydrogenous precipitation of heavy metals cannot cause the anomalous heat-flow distribution in the Bauer Deep. Areas of high heat flow could be caused by hot hydrothermal fluids moving from the basaltic crust into the sedimentary column. The uniformly thin sediment cover in the basin means that such heat would be conducted to the surface in a relatively short time (<100,000 yr), (R. N. Anderson *et al.*, unpublished). Low heat flow would follow this initial period of high flux since significant heat would have been removed from the oceanic crust[12]. Therefore, hydrothermal circulation can account for the bimodality of observed measurements in the Bauer Deep.

The Bauer Deep is the only area of extensive metalliferous sediments so far discovered away from a mid-ocean ridge crest. It is also characterised by extremely rough topography associated with an age boundary or step marking a jump in spreading centres from the Galapagos Rise to the East Pacific Rise. The mechanics of this jump required the emplacement, 6 Myr ago of new, hot material from the ridge crest into crust at least 10 Myr old (ref. 18) at that time. The introduction of this new heat source into the area may have initiated hydrothermal circulation which had been dormant since the previous thermal event contiguous with the seafloor spreading process. Dehydration reactions driven by the reheating may have released H_2O for advection to the sediment interface and opened previously clogged cracks to allow further circulation[12]. Otherwise, the association between metalliferous sediments adjacent to a major ridge jump and the bimodal distribution of heat flow in the area must be related to different mechanisms and must be coincidental. The association of both on ridge crests seems, however, to argue against coincidence.

Additionally, JOIDES holes 320 and 321 show evidence for a cessation of hydrothermal precipitation approximately 10 Myr BP. Hart *et al.*[8] concluded that this may be related to the westward jumping of the ridge. They imply that precipitation may be generally related to proximity to the spreading process heat source.

Heat flow may prove to be an adequate exploration tool for the discovery of further metalliferous sediments on the sea floor. The process of ridge jumping is not unique to the Bauer Deep. That also occurred between 25°S and 35°S on the Nazca Plate, for example. This is perhaps an ideal place to prospect for heavy metals.

The IDOE Nazca Plate Project provided important geochemical information on composition and distribution of metalliferous sediments on the Nazca Plate. This work was funded by the National Science Foundation and the Office of Naval Research. J. Greenslate, J. Natland, R. Moberley and V. Vacquier reviewed the manuscript.

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Manipulation of restriction targets in phage λ to form receptor chromosomes for DNA fragments

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*Fragments of DNA from derivatives of phage λ having either one or two targets for R.*Eco*RI have been joined in new combinations to give biologically active phage genomes. These include two classes of deletion mutants; in one, only the DNA between the targets is deleted, but in the second the deletions are more extensive. Other fragments of DNA have been inserted into these phage chromosomes.*

RECENT advances in the biochemistry of DNA offer means of creating combinations of genetic material that cannot be achieved by genetic recombination. Biologically active DNA molecules have been reconstituted from separated, sheared halves of bacteriophage λ DNA by the successive action of λ exonuclease and polynucleotide ligase[1] and a derivative of λ DNA has been fused to an animal virus genome[2]. The highly specific restriction endonucleases[3-5] have become powerful new tools for the fragmentation of DNA molecules and the study of the organisation of these fragments within viral genomes[6,7]. Of special interest in the present context are the enzymes that make breaks a few base pairs apart in opposite strands of duplex DNA to produce fragments with short, complementary, single-stranded projections or cohesive ends[8-11]. Such DNA fragments can be joined by annealing the cohesive ends and closing the single-strand breaks with polynucleotide ligase[12].

The first restriction enzyme of this type to be described was isolated from a strain of *Escherichia coli* carrying the RI drug-resistance factor[9]. Some small, circular DNA molecules have only one target[12,13] for the RI restriction endonuclease, R.*Eco*RI[14], making them suitable receptors for R.*Eco*RI fragments of DNA from other sources. Insertion of a fragment of DNA into the single R.*Eco*RI site of plasmid pSC101 destroyed neither the tetracycline-resistance phenotype nor the ability of the plasmid to replicate, so that the recombinant DNA molecules were able to transform *E. coli*[13]. This type of experiment has important potential for cloning, and hence studying, DNA from other sources. Bacteriophage λ, with its extensively studied genetics and biochemistry could be a particularly useful alternative vehicle for applications of this sort. The DNA of phage λ, however, has five targets for R.*Eco*RI[15], so our first problem was to construct derivatives of λ whose chromosomes have only one target for this enzyme. We have made a series of such phages and shown that their DNA can be broken with R.*Eco*RI to give fragments which we have joined in new combinations, thus introducing specific deletions. We have also shown that fragments of DNA produced by the action of R.*Eco*RI can be inserted between two fragments of λ DNA having respectively the right and left ends of the phage DNA. These recombinant DNA molecules were isolated as plaques following transfection of *E. coli*.

Construction of phage with single targets for R.*Eco*RI

Treatment of phage λ DNA with R.*Eco*RI gives six fragments of DNA separable by electrophoresis in polyacrylamide or agarose gels[15]. These fragments result from breakage of the DNA within the five restriction targets whose positions on the physical map of the λ chromosome have been deduced (Fig. 1)[15] (R. W. Davies, personal communication). We noted that two of these targets, designated *srl*1 and *srl*2 according to the con-

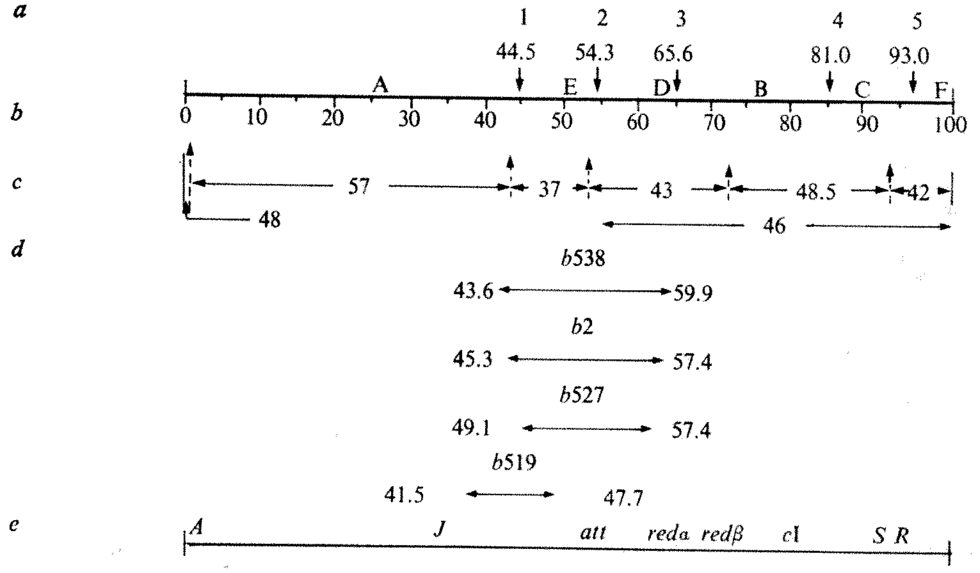
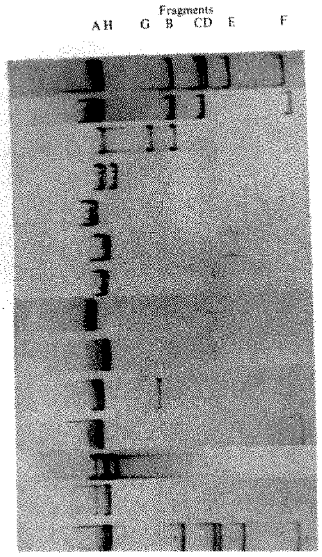


Fig. 1 Some characteristics of the λ chromosome. *a*, Positions of the targets, *srl*1–5, recognised by endonuclease R.*Eco*RI (R. W. Davies, personal communication) and location of the DNA fragments (A-F) (identified in Fig. 2) resulting from breakage of λ DNA by this enzyme. *b*, Unit length (%). *c*, The G+C content (%) of DNA fragments obtained by shearing[22]. The composition of DNA to the right of *srl*2 was calculated from that of the sheared fragments. *d*, Location of deletions[37]. *e*, Positions of some genetic markers on the physical map.[37] *A*, *J*, *red*α, *red*β, *c*I, *S* and *R* are genes; *att* is the attachment region within which recombination takes place to integrate λ into the *E. coli* chromosome.



Phage no.	*srI* Targets	Restriction coefficient	Deletion	Additional genetic markers
I		5,000		
II		400	*b*538	*c*Iam
III		80	*b*538	*c*Iam
IV		10	*b*538	*c*Iam
V		1	*b*538	*c*Iam
VI		30		*c*Iam
VII		10	*b*527	*c*Iam
I (no enzyme)				
VIII		3	*b*519	*c*Iam
IX		10	*b*538	*c*I857
X		10	*b*538	*c*Iam *S*am7
XI		7	*srI*1-3	*c*Iam
XII		5	*srI*1-2	*c*Iam
I		5,000		

Fig. 2 Electrophoretic separation of R.*Eco*RI fragments of DNA from λ^+ and various derivatives. The location on the chromosome of targets for the enzyme, of the DNA fragments, and of deletions is shown in Fig. 1. The faint band visible behind the largest fragment results from adhesion of fragments by means of the natural cohesive ends of λ DNA. Superscript *a* denotes phage with hybrid targets generated by recombination *in vitro*. Although the two fragments of DNA from phages VII, VIII, and XII were not readily resolved by gel electrophoresis under the conditions used here, they have been clearly separated by equilibrium centrifugation in Cs_2SO_4–$HgCl_2$ solutions. Phage were prepared from lysates (about 2l, titre usually 5.10^{10} p.f.u. ml^{-1}) made by infection of *E. coli* C600 in liquid culture. After two cycles of differential centrifugation (before and after treatment with RNase and DNase at 10 μg ml^{-1}) the phage were resuspended in phage buffer (KH_2PO_4, 0.3% w/v; Na_2HPO_4, 0.7% w/v; NaCl, 0.5% w/v; $MgSO_4$, 1 mM; $CaCl_2$, 0.1 mM; gelatin 0.001% w/v), CsCl was added to 41.5% w/w and the solutions centrifuged to equilibrium. The phage band was removed with a Pasteur pipette, diluted with more CsCl in phage buffer, again centrifuged to equilibrium, and the band of phage collected after puncturing the bottom of the tube. Phage were stored in CsCl solution and DNA prepared as required by extraction of a diluted, dialysed solution with freshly distilled phenol (three extractions) followed by extensive dialysis against 0.01 M Tris-HCl (*p*H 8.0) 1 mM EDTA. DNA (0.3 to 1.0 μg depending upon the number and size of fragments generated) was incubated with R.*Eco*RI, prepared essentially as described by Yoshimori[38], in 20 μl. 0.01 M Tris-HCl (*p*H 7.5) 0.01 M 2-mercaptoethanol, 0.01 M $MgCl_2$ at 37° C for 30 min and the reaction stopped by addition of excess EDTA. The necessary quantity of enzyme (about 0.1 μl) was determined by titration against varying quantities of DNA and analysis by gel electrophoresis. The digests were heated in sealed capillaries at 70° C for 10 min, cooled rapidly at 0° C, mixed with 0.1% bromophenol blue in 50% w/v glycerol (2 μl) and applied to wells in 1% w/v agarose gels[40] (approx. 40 cm × 20 cm × 0.3 cm) containing 0.4 μg ml^{-1} ethidium bromide [41] and after electrophoresis for about 16 h at a constant current of 40 mA the gels were photographed under ultraviolet light on Ilford FP4 film with a 4× red filter. For the *in vitro* recombination experiments, reaction mixtures of about 0.2 to 0.5 ml containing total DNA fragments, 10–30 μg ml^{-1}; Tris-HCl (*p*H 7.5) 66 mM; EDTA, 1 mM; $MgCl_2$, 10 mM; NaCl, 40–80 mM; dithiothreitol, 10 mM; ATP, 0.1 mM; bovine serum albumin, 0.1 mg ml^{-1}; T4 polynucleotide ligase (Miles Laboratories, Ltd), 0.5 u ml^{-1}; were incubated at 10° C for 45 min and then kept on ice for 2–10 d before sampling for transfectants.

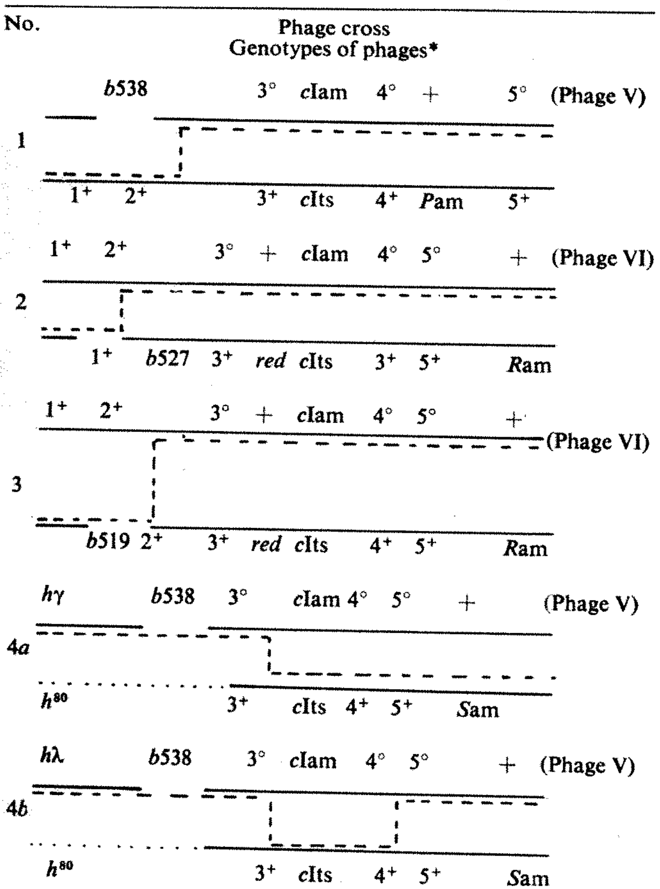
vention of Arber and Linn[16], are within that part of the central, inessential region of the phage genome which is deleted in strain *b*538[17] (see Fig. 1). Should the magnitude of restriction *in vivo*, as defined by the restriction ratio (that is the titre of non-modified phage assayed on the propagating host, divided by the titre on the restricting host), decrease as the number of restriction targets decreases[18,19] then the deletion strain *b*538 should be restricted less efficiently than λ wild-type. Furthermore, phages with even fewer restriction targets should have a selective advantage on transfer from a non-modifying host to a restricting host.

Loss of the two restriction targets in the deletion strain, b538, is indeed accompanied by a fall in the restriction ratio from 5,000 to approximately 400, concomitant with which is the predicted change in the pattern of DNA fragments observed on restriction of the DNA *in vitro* (Fig. 2). Fragments A, E and D of the wild-type strain (phage I) were replaced by a single large fragment, while fragments B, C and F were retained in the *b*538 deletion strain (phage II). We attempted, therefore, to select for mutations resulting in the loss of the remaining restriction targets. Lysates of the deletion phage (phage II in Fig. 2) were made alternately on *E. coli* strain K and on a K strain harbouring the RI plasmid[20]. After seven such double cycles the restriction ratio had dropped from around 400 to 80 and after a total of eleven cycles the phage lysate comprised mainly restriction-resistant phages. Phages with restriction ratios of 80 (phage III) and 10 (phage IV) were isolated during the course of this enrichment and purified. Gel electrophoretic analyses of R.*Eco*RI digests of their DNAs (Fig. 2) showed that phage III differed from phage II in that fragments C and F had been replaced by a larger fragment (G): we conclude that this phage had lost the right-most restriction target (*srI*5). DNA from the phage with a restriction ratio of 10 (phage IV) gave two large fragments: since fragments G and B were replaced by fragment H (Fig. 2) this phage must have retained only *srI*3 rather than *srI*4. The DNA of the restriction-resistant phage (phage V) migrates as a single band in a position indistinguishable from that of whole λ DNA molecules. From a cross (Table 1, cross 1) of the restriction-resistant phage (phage V), to a suitably marked strain of phage λ having a full complement of restriction targets, phages having only the two left-most restriction targets were isolated. These phages have a low restriction ratio (~ 30), and the DNA fragments resulting from *in vitro* restriction (phage VI, Figs 2 and 3ii) included fragment E, as expected if phage VI had acquired *srI*1 and *srI*2. Attempts to isolate spontaneous mutants lacking either *srI*1 or *srI*2 were unsuccessful because the selection favours deletions that remove both restriction targets. Phages having only *srI*1 (phage VII) or only *srI*2 (phage VIII) were therefore isolated as recombinants from crosses of phage VI (see Table 1) to the appropriately marked deletion strains *b*527 and *b*519. The agarose gel does not readily separate the two large fragments expected when the DNA of either phage VII or phage VIII is restricted (Fig. 2) but these fragments were readily separated on caesium sulphate–mercuric chloride density gradients (data not shown). Phages having only *srI*4 (phage IX) or only *srI*5 (phage X) were isolated from a cross (Table 1, cross 4) by virtue of the close linkage of *srI*4 to *cI* and of *srI*5 to gene *S*. The restricted DNA from phage IX shows two fragments including that (G) expected as the sum of fragments F and C, while the restricted DNA from phage X again shows two fragments, the smaller of which is fragment F (Fig. 2).

Separation and joining of DNA fragments

Fragments of DNA can be separated by gel electrophoresis but this method is inadequate for the preparative resolution of the larger fragments (Fig. 2). Instead, we turned to equilibrium centrifugation in caesium sulphate solutions containing mercuric chloride[21] for this gave good separation of sheared fragments of λ DNA, enabling them to be placed in order on the λ chromosome[22]. These results (Fig. 1) show that the three fragments arising from breakage of phage VI DNA with R.*Eco*RI would have quite different densities, as would the corresponding two fragments of phage IV DNA. Fragments of DNA from these phages were separated as predicted (Fig. 3i and ii) and recovered quantitatively.

Table 1 Selection of recombinant phages


No.
Phage cross
Genotypes of phages*
b538 3° cIam 4° + 5° (Phage V)
1
1+ 2+ 3+ cIts 4+ Pam 5+
1+ 2+ 3° + cIam 4° 5° + (Phage VI)
2
1+ b527 3+ red cIts 3+ 5+ Ram
1+ 2+ 3° + cIam 4° 5° + (Phage VI)
3
b519 2+ 3+ red cIts 4+ 5+ Ram
hγ b538 3° cIam 4° 5° + (Phage V)
4a
h80 3+ cIts 4+ 5+ Sam
hλ b538 3° cIam 4° 5° + (Phage V)
4b
h80 3+ cIts 4+ 5+ Sam

No. 1: *srI*1$^+$*srI*2$^+$ (that is *b*538$^+$) *c*Iam *P*$^+$ recombinants were selected as phages of normal buoyant density giving clear plaques at 32° C on a suppressor-free (*sup*°) host; those with R†~30 were shown to have only targets *srI*1 and *srI*2 (Phage VI, Fig. 2). No. 2: *b*527 *red*$^+$ cIam *R*$^+$ recombinants were selected on a *sup*° *polA* host[29] on a medium supplemented with sodium pyrophosphate (3 mM)[17]; most recombinants have R~10 and have only *srI*1 (Phage VII, Fig. 2). No. 3: *b*519 *red*$^+$ cIam *R*$^+$ recombinants were selected on a *sup*° *polA* host[29] on a medium supplemented with sodium pyrophosphate (3 mM)[17]; most recombinants have R~4, and have only *srI*2 (Phage VIII, Fig. 2). No. 4*a*; *h*λ *b*538 *c*1857 *S*$^+$ recombinants were selected as clear plaques at 39° C on a host resistant to phi80 and carrying *sup*II, which suppresses *c*Iam but not *S*am7[32]. A recombinant having R~10 was shown to have only *srI*4 (Phage IX, Fig. 2). No. 4*b*: *h*λ *b*538 *c*Iam phages were isolated as turbid plaques at 42° C (high temperature selects against phi80 host range) on a *sup*III host after enriching for those phages (*S*am7) unable to lyse a *sup*III° host. (The unlysed cells were concentrated and the phage released by chloroform.) An *S*am7 recombinant having R~10 has only *srI*5 (Phage X, Fig. 2).

*Abbreviations used: *c*Iam=*c*Iam509; *c*Its=*c*I857, *P*am=*P*am80; *R*am=*R*am5; *S*am=*S*am7; *red*=red3; 1$^+$=*srI*1; 2$^+$=*srI*1 etc. and 1°, 2° and so on refer to mutations conferring resistance to restriction.

†R=Restriction ratio=$\frac{\text{Titre on K strain}}{\text{Titre on K/RI strain}}$

The media, methods of making and assaying phage stocks and crosses were as described previously[19,31]. The standard *rec*$^+$ host for phage crosses was QR47[33].

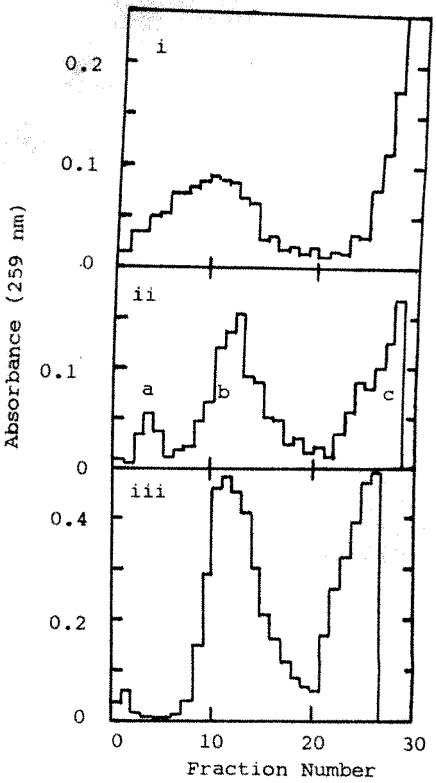


Fig. 3 Separation of DNA fragments in R.*Eco*RI digests by equilibrium centrifugation in Cs_2SO_4–$HgCl_2$ gradients. Density increases from right to left. (i) Phage IV, (ii) Phage VI, (iii) Phage XII. Solutions of DNA (100 μg in 1.0 ml 0.01 M Tris-HCl (*p*H 7.5) 0.01 M 2-mercaptoethanol, 0.01 M $MgCl_2$) were digested with R.*Eco*RI (10 μl concentrated DEAE-cellulose fraction; 37° C, 1 h), extracted with an equal volume of freshly distilled phenol and dialysed twice against 0.3 M Na_2SO_4 in 5 mM sodium borate (*p*H 9.0) and then twice against 5 mM borate (*p*H 9.0). The solutions were then heated at 65° C for 10 min, cooled in ice, weighed and their DNA concentration was determined spectrophotometrically. Mercuric chloride solution (1 mM) was then added to give an r_f of 0.22 (refs 21 and 22) and the solutions were diluted to 2.0 g with the borate buffer and Cs_2SO_4 added to 42.0% w/w. The solutions were transferred to 10 ml polypropylene centrifuge tubes, overlaid with liquid paraffin and centrifuged at 20° C at 30,000 r.p.m. for 60 h in an MSE SS65-II centrifuge, 8 × 40 Ti rotor with adaptors. Fractions of 4 drops were collected, diluted with 0.2 ml H_2O and analysed spectrophotometrically.

Our first experiments on the joining of R.*Eco*R*I* fragments of λ DNA involved the construction of phage chromosomes with specific deletions so that the phage head would be able to accommodate additional DNA. We therefore confined our attention to phages with the targets *srI*1, *srI*2 and *srI*3 because removal of the DNA between either *srI*1 and *srI*2 or between *srI*1 and *srI*3 would affect only the inessential region of the λ chromosome. Receptor chromosomes were constructed from (*a*) the left and right fragments of phage VI DNA, and (*b*) the left fragment of phage VI DNA and the right fragment of phage IV DNA. In (*a*) the mixture of fragments in an R.*Eco*RI digest of phage VI DNA was used but in (*b*) the isolated fragments of each DNA were used. The mixtures of fragments were stored in the cold for 2 d under conditions favouring the annealing of the cohesive ends of R.*Eco*RI breaks[12] and incubated with phage T$_4$ polynucleotide ligase[23]. After storage at 0° C for 2–10 d, samples were taken from the reaction mixtures for transfection of *E. coli* (ref. 24 and A. E. Jacob and S. J. Hobbs, personal cummunication) and the phages from the resulting plaques were isolated and investigated.

In a second series of experiments R.*Eco*RI fragments of DNA were inserted into the genomes of our receptor phages and into that of phage IV, which possesses only *srI3* and carries the *b*538 deletion. The central fragment of phage VI DNA, isolated from a caesium sulphate gradient (Fig. 3ii), and a DNA fragment carrying part of the *trp* operon of *E. coli*, isolated by agarose gel electrophoresis of an R *Eco*RI digest of DNA (with a ^{32}P marker) from a λ*trp* phage (λ*trp*13, which had incorporated the bacterial *trpD* and *E* genes without deleting *srI3*)[20], were used.

Annealing of DNA fragments with cohesive ends would be expected to yield a range of products, each convertible into single DNA molecules, linear or circular, by polynucleotide ligase. SV40 DNA, for example, contains one target for R *Eco*RI and gave monomeric and concatenated linear and circular molecules in these reations[12]. With λ DNA the situation is more complex because reactions will occur between the natural cohesive ends of the molecule[25] as well as the cohesive ends generated by R *Eco*RI. The length and composition of the natural cohesive ends[26] relative to those created by R *Eco*RI[9] is such that the former would cohere preferentially. The reaction products used for transfection will, therefore, comprise a complex population of DNA molecules, though many of these would not yield viable phage, either because they are too large to be packaged or because they lack essential parts of the λ genome[27]. In the experiments described here the yield of transfectants from the products of ligase reactions varied from 20 to 120 phage per μg DNA in the transfection mixture, compared with about 10^3 to 10^4 phage per μg from the corresponding DNA before restriction. (In subsequent experiments yields of transfectants from ligase reactions have been increased to about 5,000 per μg DNA and about 5 × 10^4 per μg from unrestricted DNA.) Omission of ligase reduced yields of transfectants from restricted DNA to about 1%, some of which appeared to result from joining of fragments *in vivo*.

Characteristics of the *in vitro* recombinants

(*a*) Receptor genomes. The three fragments from phage VI DNA could be joined to reconstitute parental phages or the two large fragments could be joined to give a phage lacking the DNA normally present between the two restriction targets (that is, ▽ *srI1-2*). Only one of twenty progeny made from the fragments of phage VI had the full complement of DNA, and could have resulted from the joining of all three fragments. The remaining 19 isolates showed an increased resistance to chelating agents[17], as expected for phages with deletions. Eighteen of these nineteen phages had a restriction ratio characteristic of phage with a single restriction target. The remaining phage was restriction-resistant and could have resulted from joining of fragments by means other than the short cohesive ends generated by R *Eco*RI. The deletion within this phage must extend through *srI1* and *srI2* and yet this and the other phages were able to integrate into the *E. coli* chromosome to give stable lysogens as shown by their ability to form stable, immune colonies[28]. (The alternative explanation that we have selected a deletion phage present in the original phage preparation seemed unlikely since the lysate had been purified by two cycles of equilibrium centrifugation in CsCl solution, furthermore most deletion mutants of phage λ are integration defective[17].) The conclusion that the essential features of the attachment region are right of *srI2*, is strengthened by the fact that the new deletion phages (▽ *srI1-2*) showed normal site-specific recombination in phage crosses (Table 2). Gel analyses of the restricted DNA of one of these ▽ *srI1-2* phages (phage XII) confirmed that fragment E was missing: the two large fragments present were separable in a caesium sulphate–mercuric chloride gradient (Fig. 3iii). The buoyant density of phage XII indicates a deletion equivalent to 9.7% of the λ chromosome.

Phage formed by joining the left-hand fragment of phage VI and the right-hand fragment of phage IV lack the DNA normally present between targets *srI1* and *srI3* (that is ▽ *srI1-3*). The five phages isolated from such an experiment all had a low restriction ratio (~ 7), showed an increased resistance to chelating agents, failed to integrate into the *E. coli* chromosome and failed to form plaques on a *polA* host. The latter property[29] is characteristic of all recombination-deficient (*red*$^-$) strains of phage λ and is consistent with the deletion of at least part of the *red* genes. Crosses of the new deletion phages to appropriately marked *red*$^-$ strains of phage λ (Table 3, line 4) confirm that ▽ *srI1-3* phages are defective in the *red*α gene but not in the *red*β gene (see Fig. 1). These data provide genetic evidence that *srI3* is either within the *red*α gene or between *red*α and *red*β. Gel electrophoreses of the restricted DNA of one of the ▽ *srI1-3* phages (phage XI) confirm the presence of the two predicted DNA fragments and the buoyant density of phage XI indicates a deletion of 20.7% of the λ chromosome.

(*b*) Phages made from three fragments. The purified fragment E from phage VI was annealed with the restricted DNA from phage IV, which has only target *srI3*, and the mixture treated with ligase. Since phage IV has the *b*538 deletion (see Fig. 1), it lacks the DNA of fragment E and, if *srI3* is within the *red*α gene, the insertion of DNA at this site will produce a *red*$^-$ phage. We isolated 78 transfectants from the reaction mixture and detected six defined as *red*$^-$ by their failure to form plaques on a *polA* host[29]. All six *red*$^-$ phages were characterised as

Table 2 Site specific recombination in phage crosses

Cross*					Frequency of site specific recombination (Twice the frequency of turbid (c^+) J^+R^+ recombinants)
*J*am6	*att*$^+$	*red*3	c^+	+	1.33%
+	*att*$^+$	*red*3	*c*I857	*R*am5	
*J*am6	*att*$^+$	*red*3	c^+	+	0.024%
+	*b*538 (*att*▽)	*red*3	*c*I857	*R*am5	
*J*am6	*att*$^+$	*red*3	c^+	+	1.46%, 1.37%, 1.44%**
+	*srI1–2*	*red*3	*c*I857	*R*am5	

* These crosses of *red*$^-$ phages were made in a *recA* host (QR48)[33].

** Three different ▽ *srI1–2* recombinants were used, one of which was restriction resistant and another was a recombinant made from purified fragments.

Table 3 The *red* functions of *in vitro* recombinants

Test phage	Recombination frequency (%) between immunity and gene R expressed as twice the frequency of $i^{4*}R^{+}$ recombinants	
	(*a*) Cross to *red*α i^{4}*R*am5	(*b*) Cross to *red*β i^{4}*R*am5
λ ▽ *srI*1–2 $i^{\lambda}R^{+}$ (*red*⁺)	7 2	2 1
λ *bio*69 $i^{\lambda}R^{+}$ (*red*α⁻)	0 02	1 6
λ *red*3 $i^{\lambda}R^{+}$ (*red*β⁻ polar on *red*α)	1 4	0 02
λ ▽ *srI*1–3 $i^{\lambda}R^{+}$ †	0 02, 0 02	2 3, 2 4
λ *b*538 *srI*3 *red*⁻R^{+} ‡	0 02, 0 01, 0 01, 0 02, 0 02, 0 02	2 8, 3 0, 2 3, 2 8, 2 9, 2 1

* The abbreviation i^{4} is used for the immunity of phage 434
† 2 different *srI*1–3 deletion phages
‡ The six *red* phages resulting from insertion of DNA at *srI*3
The Red phenotypes of the phages were deduced from the frequencies of $i^{4}R^{+}$ recombinants generated in crosses to a *red*α phage (λ *bio*69 i^{4}*R*am5) and to a *red*β phage (λ *red*β113 i^{4}*R*am5)[39] All crosses were made in the *recA* host, QR48[33], and the recombinants selected on a sup^{0} (λ i^{λ}*R*am5) strain of *E coli*

*red*α⁻*red*β⁺ (Table 3, line 5) Five of these six resembled the original phage IV in having a restriction ratio of around 10, as expected for a phage with only *one* restriction target the remaining *red*⁻ phage (Phage XIII) had a restriction ratio of 80, suggesting two restriction targets This phage meets the predictions for a recombinant in which the whole of fragment E had been inserted at *srI*3 Gel electrophoresis of the restriction products of the DNA from phage XIII (Fig 4) shows two large DNA fragments like those of phage IV and a third fragment of the same size as fragment E

One of the other five phages was investigated further This phage (Phage XIV) had a buoyant density less than that of the receptor phage (Phage IV) and analysis by gel electrophoresis of the restricted DNA from this phage showed one fragment moving in the same position as the smaller fragment of the receptor DNA and a slower fragment moving slightly faster than the larger fragment of the receptor genome The formation of this and the other *red*⁻ phages with only one restriction target can be explained if fragment E joined to one end of the receptor chromosome via its cohesive end and then at the other end via an illegitimate recombination event[30] In the case of phage XIV the illegitimate recombination event was associated with the deletion of more than 10% of the phage DNA This explanation for the origin of those *red*⁻ phages having a single restriction target is supported by a control experiment in which fragment E was not added to the receptor DNA and here the ten *red*⁻ phages isolated were all restriction resistant

A fragment of DNA containing the *trpD* and *E* genes of the *trp* operon of *E coli* together with their operator and promoter was isolated and purified from an $h^{80}i^{\lambda}$*cI trp*-transducing phage (λ*trpI*3)[20] This fragment was fused to the restriction products of the receptor phage ▽ *srI*1-2 (Phage XII) and among 120 transfectants we detected[31] one $h^{\lambda}i^{\lambda}$ *clam* phage having the *trpD* and *E* genes This λ*trp* phage expresses the *trp* genes from the *trp* promoter and from a λ promoter (N E M and W J Brammar, unpublished observation) Like four of the five recombinant *red*⁻ phages described above, this phage has a low restriction ratio ($R \sim 10$) and has not incorporated the whole of the DNA fragment In fact, the phage has become integration defective consistent with loss of DNA to the right of *srI*2 The buoyant density of the new λ*trp* phage indicated that it has about 3% more DNA than λ wild type Had the whole fragment been incorporated into the receptor genome then a phage with at least 10% more DNA than λ wild type would be expected At least two other phages had acquired extra DNA but did not contain the *trp* genes

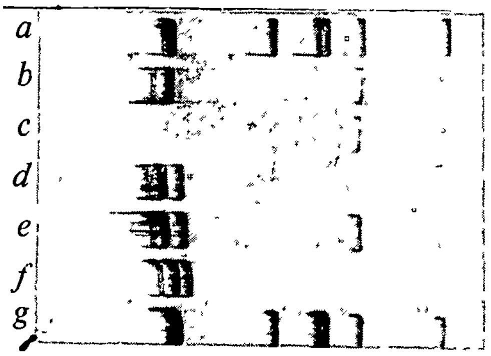


Fig 4 Agarose gel electrophoresis of R *Eco*RI digests of DNA used in and obtained from recombination experiments *in vitro* Experimental details were the same as described in the legend to Fig 2 (*a*) Phage I, (*b*) Phage VI, (*c*) Fragment E purified from phage VI (peak a from Fig 3*b*), (*d*) Phage IV, (*e*) Phage XIII, (*f*) Phage XIV (Phages XIII and XIV are the recombinant phages), (*g*) Phage I The faint, slowest-moving band visible in (*b*), (*d*), (*e*) and (*f*) results from adhesion of the large fragments of DNA via their normal cohesive ends

Practical implications

Our results show that the phage λ genome can be used as a receptor for genetic material that cannot be incorporated by conventional genetic techniques The biochemical approach used provides a convenient means of cloning DNA, but more importantly it lends the extensive genetic and biochemical experience acquired with phage λ[34] to the study of the additional genetic material This DNA can be inserted into the phage chromosome so that its expression is controlled autonomously, or by the phage regulatory systems[12], when advantage can be taken of the efficient λ promoter, P_L, and *N*-gene protein which allows RNA polymerase to read through transcriptional stops[43] By appropriate choice of the phage genotype the yields of gene products can be greatly enhanced[35], experiments with λ*trp* phages, for example, have shown that the five enzymes of the *trp* operon can be boosted to comprise as much as 50% of the soluble protein of the host cell (A Moir and W J Brammar, personal communication) The insertion of appropriate DNA fragments into marked receptor phages offers new approaches for extending the biochemical genetics of eukaryotic systems We have shown that eukaryotic DNA can be inserted into the λ receptor phages (unpublished results) It has been established that eukaryotic DNA can be transcribed within the *E coli* cell[36], but it is not known whether a eukaryotic messenger RNA is translated

We expected that transfectant phages would result from covalently linked fragments of DNA as either linear or circular chromosomes[24] because a linear molecule could circularise by joining of free cohesive ends after its entry into the bacterial cell Our results suggest that the bacterial cell may provide an alternative way of joining two non-homologous regions of DNA and in so doing generate deletions Further experiments have proved that deletion phages are indeed created rather than merely selected (N E M and K M , unpublished observations) and they confirm the use of this method for the production of localised deletions Illegitimate recombination events were first suspected because restriction targets were lost, those deletions generated in the region of *srI*2 have been characterised further as integration proficient, integration defective, or integration and recombination defective (K Borck and N E M , unpublished observation) Lai and Nathans[41] have detected an analogous class of deletion mutants following the *in vivo* association of restriction fragments of SV40 DNA and have made elegant use of these deletions to further the genetic analysis of the SV40 virus

Insertion of DNA fragments into a receptor chromosome *via* illegitimate recombination destroys the RI restriction target, and can therefore be a disadvantage if one is concerned with the recovery of a cloned DNA fragment It does provide, however, a means of incorporating part of the DNA from a fragment that is too large to be accommodated within the phage genome Removal of the DNA between *srI*1 and *srI*3 in the presence of a deletion between genes *P* and *Q* (*nin*R5)[45] eliminates 27% of the λ DNA Since phage λ can accommodate about 10% more than its normal complement of DNA these deletions permit the insertion of DNA equivalent to about 15 genes Although we have not used them in these experiments, powerful methods are available for selecting only those phages that have gained DNA

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Glutamine synthetase and ammonium regulation of nitrogenase synthesis in *Klebsiella*

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Glutamine synthetase seems to act as a positive control element for nitrogenase synthesis in a way similar to that already proposed for histidase synthesis in Klebsiella *Studies along lines opened up by these findings may provide answers to many of the problems in establishing nitrogen fixation in non-leguminous plants*

IN both fungi and bacteria, synthesis of enzymes involved in the utilisation of nitrogen sources such as amino acids, purines, nitrate and nitrite, is normally repressed when sufficient ammonium nitrogen for growth is provided in the culture medium Arst and Cove suggested that ammonium repression of all such enzymes in *Aspergillus nidulans* was effected by a single mechanism the *areA* gene product, as yet unidentified biochemically, seems to be essential for enzyme synthesis but is absent, or cannot function, in the presence of NH_4^+ Prival, Brenchley and Magasanik[2,3] have studied utilisation of histidine and regulation of histidase and other enzymes of the *hut* operons in *Klebsiella aerogenes* Histidine can serve as nitrogen source or carbon source, histidase is subject to both ammonium repression and glucose (carbon–catabolite) repression and the relief of either derepresses histidase synthesis (see ref 2) Derepression of histidase synthesis under limiting nitrogen is independent of cyclic AMP but requires high levels of glutamine synthetase, mutants lacking glutamine synthetase fail to derepress synthesis of histidase under these conditions[3] Moreover, in mutants which are constitutive for glutamine synthetase, histidase and proline oxidase (the first enzyme of proline catabolism) escape from ammonium repression[3] Purified unadenylylated glutamine synthetase, but not the fully adenylylated enzyme, activates correct transcription of *hut* DNA *in vitro*, glutamine synthetase is as effective in this respect as cyclic AMP plus catabolite activating protein (CAP) and has been proposed therefore as a positive control

Table 1 Growth of *K pneumoniae* M5a1 and mutant RT4 on various compounds as sole N source

	Nitrogen-source	M5a1	RT4
a	$(NH_4)_2SO_4$	+++++	+−++
	Glutamine	++++	+++
	Asparagine	++++	+
	Glutamate	++	−
	Aspartate	+++	++
	Alanine	+++	+++
	Arginine	++	−
	Histidine	+++	−
	Proline	++	−
	Nitrate, sodium	+++++	−
	Nitrite, sodium	++++	−
	Urea	+++++	+++
	Adenine	++++	−
	Hypoxanthine	+++	−
	Xanthine	++	−
b	N_2	+++	−
c	Casein hydrolysate	+++	+++

Nutrient broth cultures of M5a1 and RT4 were centrifuged, washed, resuspended in saline-phosphate and plated out on minimal medium containing, g l^{-1}, 20 glucose, 3 40 KH_2PO_4, 12 06 K_2HPO_4, 0 025 $Na_2MoO_4\,2H_2O$, 0 025 $FeSO_4$, 0 1 $MgSO_4$, supplemented where indicated with 1 g of nitrogen source *a*, Growth was estimated visually after 48 h at 30° C in air on a scale ranging from − (no growth) to +++++ (very good growth) *b*, Growth was estimated similarly after 5 d incubation at 30° C in an anaerobic bag[12] with a throughput of N_2/CO_2 (99 1) No nitrogen source was included in the medium *c*, Growth estimated from overnight liquid cultures grown under argon

element for transcription of *hut* DNA[4]

Nitrogenase synthesis in *Klebsiella pneumoniae* is repressed by NH_4^+ (ref 5), there is no evidence for glucose repression and this is reasonable since N_2, unlike histidine, can serve only as a nitrogen source Here I report (i) the characterisation of a mutant of *K pneumoniae* which was altered in its regulation of glutamine synthetase, had lost the ability to grow on a large number of compounds as sole nitrogen source and failed to synthesise both nitrogenase components, (ii) the transfer of nitrogen fixation (*nif*) genes to glutamine synthetase-constitutive mutants of *K aerogenes* resulting in constitutive expression of *nif* These results strongly implicate glutamine synthetase as being involved in ammonium regulation of nitrogenase synthesis

Pleiotropic mutation which blocks nitrogenase synthesis

Mutant strain RT4 was obtained by mutagenesis of *K pneumoniae* M5a1 with IPMS (isopropylmethane sulphonate)[6] followed by selection with ampicillin and D-cycloserine for glutamine auxotrophs RT4 grew well on glutamine-supplemented minimal medium, grew well, but with a longer lag, on unsupplemented minimal medium (with $(NH_4)_2SO_4$ as sole nitrogen source) and failed to grow on NH_4^+-free medium (NFM) with histidine, nitrate or N_2 as sole nitrogen source The mutation was, in fact, extremely pleiotropic resulting in loss of the ability to utilise many nitrogen sources (Table 1) and was superficially very similar to the *asm*⁻ mutation described by Nagatani, Shimizu and Valentine[7], such *asm*⁻ mutants produce low levels of glutamate synthase[7 10] (glutamine-amide-2-oxoglutarate amino transferase) This enzyme is responsible for the second step in the glutamine synthetase–glutamate synthase pathway, the primary route of NH_4^+-assimilation in *Klebsiella* grown with low NH_4^+ or poor nitrogen sources[7–10] Although *asm*⁻ mutants fail to grow on N_2 as sole nitrogen source, Streicher *et al*[11] mentioned that nitrogenase, as determined by the acetylene reduction test, is induced, nitrogenase levels are comparable to the wild-type level in aspartate-supplemented medium RT4, however, showed no acetylene reduction in Pankhurst tube tests[13,14] on NFM with either vitamin-free casein hydrolysate (160 μg ml^{-1}) or L-glutamine (100 μg ml^{-1}) as nitrogen source It was possible that NH_4^+ produced from these nitrogen sources was not readily assimilated and therefore accumulated to repress nitrogenase synthesis In neither case, however, was NH_4^+ detectable in the culture supernatant at the end of the test

Comparison of nitrogenase, glutamine synthetase, glutamate synthase and glutamate dehydrogenase activities in RT4 and M5a1 confirmed that RT4 was not *asm*⁻ (Table 2) Cultures were grown in conditions which allowed derepression of nitrogenase synthesis yet in which growth was independent of nitrogen fixation and in which Nif⁺ revertants of Nif⁻ RT4 had no selective advantage Glutamate synthase activity in RT4 was very similar to that in M5a1 But, glutamine synthetase activity ($-Mg^{2+}$) was 40% lower and below the 0 7 units of activity required for the escape of histidase synthesis from ammonium repression[3] Glutamate dehydrogenase was 50% higher Nitrogenase activity was again absent from whole organisms of RT4 When ATP + dithionite-dependent nitrogenase activities were assayed in anaerobically prepared cell-free extracts of RT4, both component activities were absent (Table 3) Brenchley, Prival and Magasanik[10] showed that levels of glutamine synthetase and glutamate dehydrogenase varied inversely in *Klebsiella aerogenes* During growth on low NH_4^+, glutamine synthetase level is high, glutamate dehydrogenase low, ammonium assimilation through glutamine synthetase and glutamate synthase is favoured When NH_4^+ is in excess, glutamine synthetase is low, glutamate dehydrogenase high ammonium assimilation can proceed through glutamate dehydrogenase Brenchley *et al*[10] suggested that glutamine

Table 2 Nitrogenase, glutamine synthetase (GS), glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH) activities of M5a1, mutant RT4 and revertants of RT4

Strain	Relevant phenotype	Nitrogenase*	GS†			GOGAT‡	GDH‡
			$-Mg^{2+}$	$+Mg^{2+}$	$\%+Mg^{2+}/-Mg^{2+}$		
M5a1	Hut⁺ Nif⁺	38	0 99	1 34	135	92	79
RT4	Hut⁻ Nif⁻	0	0 60	1 02	170	97	125
RT48	Hut⁺ Nif⁺	24	0 73	1 13	155	—	—
RT49	Hut⁺ Nif⁻	0	0 64	1 06	166	—	—

Cultures were grown at 28° C on NFM supplemented with 1 2 mg casein hydrolysate ml^{-1} and sparged with argon When a culture reached approximately 0 25 mg bacterial protein ml^{-1} (40 EEL Spectra units at 540 nm) a sample was removed and assayed for whole-cell acetylene reduction[5] Cell-free extract was prepared from the rest of the culture as described by Prival *et al*[3] except sonication was with a Dawe Soniprobe for 4 × 15 s at 2 5 A with 1 min cooling intervals Glutamine synthetase activities were measured by 'transferase' assays[15] with and without 60 mM $MgCl_2$ (ref 3) Assaying in the absence of Mg^{2+} gives a measurement of total glutamine synthetase (both adenylylated and unadenylylated subunits are active) whereas assaying in the presence of Mg^{2+} gives a measurement of unadenylylated enzyme which is believed to be the biosynthetically active form *in vivo* $MgCl_2$, although it inhibits adenylylated subunits, appears to stimulate activity of unadenylylated glutamine synthetase in crude extracts (see ref 3) Activities measured with Mg^{2+} therefore overestimate *in vivo* glutamine synthetase activity '$\%+Mg^{2+}/-Mg^{2+}$' overestimates % unadenylylated enzyme Glutamate synthase and glutamate dehydrogenase activities were measured at 20° C by following NADPH oxidation at 340 nm (ref 9) with a Unicam SP1800 spectrophotometer Glutamate synthase activities here have been corrected by subtracting 10% of the measured glutamate dehydrogenase activity[10]

*nmol C_2H_2 reduced per min per mg bacterial protein

†μmol γ glutamylhydroxamate formed per min per mg protein

‡nmol NADPH oxidised per min per mg protein

— = not determined

synthetase repressed synthesis of glutamate dehydrogenase, thus the higher level of glutamate dehydrogenase in RT4 (Table 2) may be a consequence of an inability to derepress full synthesis of glutamine synthetase

Glutamine synthetase levels in RT4 and M5a1 were compared in extracts from cultures grown under both depressive conditions (glutamine as sole nitrogen source) and repressive conditions (glutamine and NH_4^+ as nitrogen sources) RT4 constitutively synthesised glutamine synthetase as a level intermediate between repressed and derepressed M5a1 (Table 4) It follows that the mutation in RT4 must directly affect regulation of glutamine synthetase synthesis, a mutation causing increased intracellular NH_4^+ production by some other pathway would explain a low level of glutamine synthetase when derepressed, but not the high level when repressed Glutamine synthetase activities in M5a1 were comparable to those reported by Prival *et al*[3] in *K aerogenes* MK-53 In agreement with these authors, the adenylylation of glutamine synthetase was increased to compensate for overproduction (see RT4 grown with NH_4^+ and L-glutamine, Table 4) Moreover, glutamine synthetase was more unadenylylated in RT4 grown on L-glutamine where overall levels of enzyme were lower than in M5a1

Table 3 Nitrogenase activities of M5a1 and RT4

Strain	Whole cell	Extract	I*	II†
M5a1	31	28	37	30
RT4	0 0	0 0‡	0 0	0 0

Activities are expressed as nmol C_2H_2 reduced per min per mg protein Cultures were grown and whole-cell activities measured as described under Table 2 Cell-free extracts were prepared anaerobically from spheroplasts by combined osmotic shock and Triton-X-100 lysis (Christina Kennedy, personal communication) ATP+dithionite-dependent nitrogenase activity was assayed in extracts as described by Eady *et al*[16] 0 5–1 0 mg of protein was used per assay

*Component I (Fe-Mo protein) activity 50 μg purified component II added to assay

†Component II (Fe protein) activity 50 μg purified component I added to assay

‡When RT4 extract was assayed in the presence of both components I and II, no inhibition of the activity of the added purified nitrogenase components was observed lack of nitrogenase activity in RT4 was not a consequence of enzyme inhibition

Spontaneous 'revertants' of RT4 were obtained at a frequency of about 1 in 10^8 organisms by plating on NFM or NFM supplemented with 1 mg L-histidine or KNO_3 per ml Clones obtained on NFM-histidine were either prototrophic or revertants which grew on histidine as sole nitrogen source but not on nitrate or N_2 Similarly, clones obtained on NFM-nitrate were either prototrophic or revertants which grew on nitrate but not on histidine or N_2 But, all of 62 clones obtained on N_2 were prototrophic, repeated attempts failed to produce a Nif^+ revertant which did not grow on histidine or nitrate Glutamine synthetase activities of typical RT4 derivatives are shown in Tables 2 and 4, RT48 was a typical prototrophic Hut^+ revertant with wild-type glutamine synthetase activities and regulation, RT49 had regained only the ability to grow on histidine as sole nitrogen source and showed glutamine synthetase activities like RT4 Apparently derepression of *hut* enzymes had become independent of high levels of glutamine synthetase in this strain

Glutamine auxotrophs of *K aerogenes* have lesions which map at two separate loci, *glnA* and *glnB* (ref 3) Most revertants of a *glnB* strain produce glutamine synthetase constitutively, these revertants ($GlnC^-$ strains) have a second mutation at a site cotransducible with *glnA* (ref 3) An F′ *nif-his* plasmid (FN68) which also confers carbenicillin-resistance[17] was transferred to RT4 from *Escherichia coli* JC5466 (provided by Dr R A Dixon) Nitrogenase activity was not restored to RT4 by this means so, unlike most *nif* mutations[11] the mutation in RT4 did not appear to be linked to *his*. The map position of the RT4 mutation is currently being sought, this work will be reported in a subsequent communication

Table 4 Regulation of glutamine synthetase in M5a1, RT4 and derivatives

Strain	Composition of medium		Glutamine synthetase		
	NH_4^+	L-Gln	$-Mg^{2+}$	$+Mg^{2+}$	$\%+Mg^{2+}/-Mg^{2+}$
M5a1	−	+	1 42	0 43	30
M5a1	+	+	0 24	0 20	83
RT4	−	+	0 62	1 04	168
RT4	+	+	0 61	0 27	44
RT48	−	+	1 17	0 42	36
RT48	+	+	0 27	0 22	81
RT49	−	+	0 63	0 96	152
RT49	+	+	0 60	0 25	42

$(NH_4)_2SO_4$ and L-glutamine were used at 1 g^{-1}, glucose at 5 g^{-1} Overnight cultures, grown up under air on an orbital shaker, were suitably diluted into fresh medium, grown for 2–3 doublings and harvested from log phase Extracts were prepared and glutamine synthetase assayed as described under Table 2

Constitutive synthesis of nitrogenase

Prival *et al*[3] showed that $GlnC^-$ strains which are fully constitutive for glutamine synthetase produce depressed levels of histidase and proline oxidase in the presence of NH_4^+, similar strains derived from nitrogen-fixing *K pneumoniae* M5a1 might be constitutive for nitrogenase synthesis If the RT4 mutation was in the *glnB* site, $GlnC^-$ strains might be found among revertants of RT4 Over 80 prototrophic clones obtained by reverting RT4 to a Hut^+ or Nif^+ phenotype were checked for the production of a yellow-brown pigment when grown on tryptophan plus NH_4^+ as nitrogen sources Pigment production under these conditions is characteristic of $GlnC^-$ *K aerogenes*[3] No $GlnC^-$ revertant of RT4 was identified by this means either the test was not applicable to *K pneumoniae* M5a1 or mutation to $GlnC^-$ does not repair RT4 Additionally, 25 out of 25 Nif^+ revertants of RT4 tested failed to reduce acetylene when anaerobically grown in the presence of 1 mg $(NH_4)_2SO_4$ per ml

K aerogenes strain MK-53 and its $GlnC^-$ derivatives MK-94 and MK-142 (see ref 3) do not naturally fix nitrogen The F′ *nif-his* factor (FN68) which carries the *nif* genes of *K pneumoniae* M5a1 (ref 17) was therefore introduced into these strains to determine the effect of constitutive synthesis of glutamine synthetase on expression of *nif* Significantly, Nif^+ His^+ exconjugants obtained by introducing the F′ *nif* into his^- derivatives of the GlnC *K aerogenes* strains MK-94 and MK-142 retained 10–20% of their derepressed level of nitrogenase activity (Table 5) when grown on NH_4^+ This partially constitutive synthesis of nitrogenase was specifically associated with the $GlnC^-$ character since it was not shown by *K aerogenes* MK-53 (FN68) or *K pneuomoniae* M5a1 Mutants of *Azotobacter vinelandii* which show 25% of their derepressed level of nitrogenase in the presence of ammonium have been described[18] but have not been characterised with respect to glutamine synthetase

Glutamine synthetase and nitrogen fixation

The possibility that synthesis of both nitrogenase and glutamine synthesis is positively regulated by an unidentified factor which is defective in RT4 and unaffected by NH_4^+ in $GlnC^-$ strains cannot be ruled out completely But it is difficult to see how a common defective regulator in RT4 could produce both low constitutive synthesis of glutamine synthetase and no synthesis of nitrogenase It seems most likely, therefore, that glutamine synthetase itself is required as a positive control element for nitrogenase synthesis in the same way as has been proposed for histidase synthesis in *Klebsiella*[3,4], a direct demonstration of activation of transcription of *nif* by glutamine synthetase *in vitro* is still required That nitrogenase synthesis only partially

Table 5 Expression of *nif* in GlnC$^-$ (glutamine synthetase-constitutive) hosts

Organism	Strain	Derivation Mating performed	 Character selected	Relevant phenotype	Nitrogenase activity (nmol C_2H_2 reduced min^{-1} mg^{-1}) Grown on NH_4^+	 Aspartate
K pneumoniae	M5a1	—	—	GlnC$^+$	<0 01	39-55*
K aerogenes	MK-53 (FN68)	MK-53 × JC5466 (FN68)	Carbenicillin resistance	GlnC$^+$	<0 01	
K aerogenes	MK-94 *his* (FN68)	MK-94 *his* × JC5466 (FN68)	His$^+$	GlnC$^-$	6 0	
K aerogenes	MK-142 *his* (FN68)	MK-142 *his* × JC5466 (FN68)	His$^+$	GlnC$^-$	7 5	

K aerogenes MK-53, MK-94 and MK-142 (≡MK-140) are published strains[3] MK-94 *his* and MK-142 *his* were derived by IPMS mutagenesis and subsequent antibiotic selection for histidine auxotrophs For matings using *E coli* JC5466 (FN68) as donor, 0 1 ml of overnight broth cultures of both donor and recipient were spread together on nutrient agar After 6–7 h organisms were scraped off, resuspended in saline-phosphate buffer at 10^8–10^9 organisms ml^{-1} and 0 1 ml plated out on selective medium The Nif$^+$ *K aerogenes* hybrids and *K pneumoniae* M5a1 were grown under a stationary argon gas phase on NFM supplemented with 1mg $(NH_4)_2SO_4$ or aspartate ml^{-1} When a culture reached 0 15–0 20 mg bacterial protein ml^{-1} a sample was removed and assayed for nitrogenase activity (see legend to Table 2)
*Dependent on age of culture

escapes ammonium repression in GlnC$^-$ strains may reflect a specific requirement for highly deadenylylated glutamine synthetase GlnC$^-$ strains contain derepressed levels of glutamine synthetase in the presence of excess NH_4^+ but the enzyme is more adenlylyated than under nitrogen limiting conditions (see ref 2) If the adenylylating 'cascade' system for glutamine synthetase[19,20] can be blocked by mutation, such a mutation in a GlnC$^-$ strain may produce fully constitutive nitrogenase synthesis

If glutamine synthetase does exert a direct effect on *nif* expression, proposed transgenosis[21] of repressible *nif* genes from *Klebsiella* to plants would require homologous glutamine synthetase structural and regulatory genes to be transferred along with *nif* This problem could be avoided if *nif*-constitutive strains in which nitrogenase synthesis was independent of activation by glutamine synthetase could be obtained If *nif* genes are in a single operon, one might have expected to obtain this class of mutants among Nif$^+$ revertants of RT4 That such attempts failed may mean that *nif* genes in *Klebsiella* are located in more than one operon

Dunican and Tierney[22] have recently succeeded in picking up *nif* genes from *Rhizobium trifolii* thereby adding conviction to the widespread, but unsubstantiated, belief that genetic material from rhizobia codes for nitrogenase proteins in the nitrogen-fixing legume symbiosis No *Rhizobium* has been shown to fix nitrogen in the free-living state Dunican and Tierney[22], however, obtained expression of presumptive *Rhizobium nif* genes by R-factor-mediated conjugation into *K aerogenes* strain 418, naturally a non-fixer In addition, expression of *Rhizobium nif* in *K aerogenes* was repressed by NH_4^+ It is tempting to speculate that free-living rhizobia do not synthesise nitrogenase because *Rhizobium* glutamine synthetase is unable to activate transcription of *nif* DNA by *Rhizobium* RNA polymerase *R japonicum* glutamine synthetase is known to be immunologically distinct from the glutamine synthetases of the coliform bacteria, *E coli, K pneumoniae* and *Salmonella typhimurium*[23] Nitrogenase is synthesised, however, within the root nodules of leguminous plants, either the plant provides suitable 'machinery' for transcription of *nif* or changes occur in *Rhizobium* RNA polymerase during establishment of effective symbiosis, such that *nif* genes can be transcribed in the absence of activation by glutamine synthetase Possible answers to questions raised by studies on symbiotic nitrogen fixation therefore can be suggested from basic knowledge of regulation of synthesis of nitrogenase in *K pneumoniae,* such information must also greatly increase the chances of success in any attempt in the future to establish nitrogen fixation in non-leguminous crop plants

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letters to nature

Possible antineutrino pulse of extraterrestrial origin

We report an unusual event observed during our search for electron antineutrino $\bar{\nu}_e$ bursts of extraterrestrial origin[1] Our experiment uses a deep underground $\bar{\nu}_e$ burst-detector station, adjacent to the Brookhaven Solar Neutrino Observatory[2] at a depth of 4,400 metres water equivalent (m w e) in the Homestake Gold Mine in Lead, South Dakota At this station we have placed a number of large water Cerenkov counters (Fig 1) designed to detect $\bar{\nu}_e$ interactions by the Cerenkov radiation from positrons resulting from $\bar{\nu}_e + p \rightarrow n + e^+$ (ref 3)

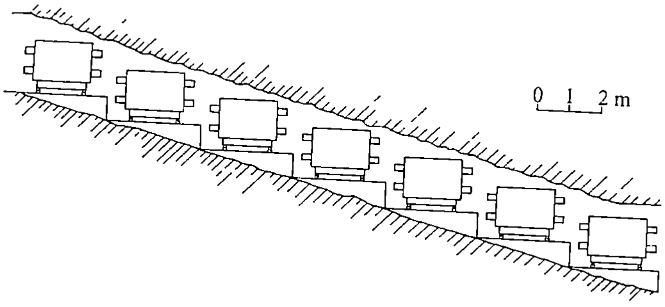

Fig 1 Arrangement of water Cerenkov counters in a tunnel 1,480 m below surface in the Homestake Mine in Lead, South Dakota The lowest right hand counter is No 1 and the highest left hand one is No 7

Each Cerenkov counter is a 2 m^3 cylinder, 1 78 m long by 1 22 m filled with deionised water containing Amino-G wave length shifter and viewed by eight photomultipliers 12 5 cm in diameter The majority logic coincidence circuit associated with each counter gives an output pulse whenever five or more of the eight photomultipliers viewing that counter give coincident signals The occurrence of either a single large amplitude pulse from any one counter or of two or more counter pulses within 0 1 s initiates a magnetic tape recording of the time distribution of the pulses in each counter, the summed photomultiplier pulse amplitude associated with each pulse and the photomultiplier pattern of each counter involved in the coincidence The time difference between pulses within an event is measured with a 20 MHz oscillator providing 50 ns time resolution for these differences

On January 4, 1974, at 17 h 16 min 48 s GMT we observed an event involving a total 24 pulses within 3 ms in six of our detectors These pulses occurred in four groups, each ~ 1 μs wide The first and second groups were separated by 640 μs, the second and third by 968 μs and the third and fourth by 928 μs The first group consisted of nine pulses distributed over the six counters, the second group involved six pulses and the third group eight pulses These three groups used up 23 of the 24 available readout channels leaving only one readout channel available for the last pulse From our detector thresholds and pulse height data, we estimate the energy of the particles involved in these pulses to be 20 MeV $\leqslant E \leqslant$ 100 MeV These pulse heights indicate that the charged particles responsible for these pulses had ranges considerably less than half of the diameter of our counters and so are unlikely to have traversed our counters

Since our readout channels were completely filled by the four recorded bursts we do not know if there were any subsequent pulses We only know that 0 8 s after the first pulse, when our transfer to tape was completed, there were no more counts, so that the pulse duration period was less than this time

Figure 2 shows the time distribution of the observed bursts and Fig 3 gives the pulse distribution within each burst Details of the burst data are given in Table 1

We have considered and eliminated the possibility that this event is due to an electronic instrumentation effect, a random accidental correlation, or a rare version of one of the well known 'local' physical phenomena The evidence we used to rule out the first possibility lies both within the event itself and in the other tape records acquired in the hours preceding and following this event A normal record containing one pulse occurred 30 s before the event and another one 9 s after the event

We observe about 300 twofold coincidences per day and about four threefolds per day within our 0 1 s time resolution These rates are in good agreement with an accidental prediction based on our 'singles' rates From these rates we would expect

Table 1 Details of the burst data

Burst	Counter 1	2	3	4	5	7
1	0 10 μs 0 60 μs	0 15 μs	0 05 μs	0 05 μs 0 45 μs 1 00 μs	0 15 μs	0 00 μs
2	639 65 μs	639 65 μs 640 00 μs *640 40* μs All readout scalers filled	639 60μs			639 70 μs
3	*1607 60* μs All readout scalers filled		1607 50 μs *1607 80* μs All readout scalers filled	*1607 60* μs All readout scalers filled	1607 65 μs 1608 00 μs	1607 45 μs *1608 10* μs All readout scalers filled
4					*2535 7* μs All readout scalers filled	

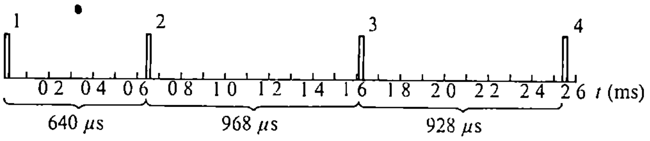

Fig. 2 Time distribution of narrow bursts of observed pulses

a 24-fold accidental correlation within 0 1 s $\approx 10^{-40}$ times per second A 3-ms correlation requirement would reduce this rate considerably

A higher rate can be obtained by considering the probability of three large angle muons traversing the apparatus within 3 ms We observe about 7 5 vertical muons per day traversing each detector and see a large angle muon going through two detectors about once in two days From these numbers we predict about one muon traversing all our detectors once a year or three muons within 3 ms $\approx 10^{-30}$ times per second Of course, this mechanism would not account for the multi-pulse structure of the bursts, nor for the small pulse heights associated with our pulses

Extensive cosmic-ray showers can have a spread in time of arrival spread of ~ 150 ns (ref 4) This spread is consistent with the time distribution of pulses within one of our bursts We observe about one cosmic ray shower per week at our apparatus but very few of them are spatially spread over more than one detector In 3 months of observation we have seen only one shower involving three detectors and none that spans more than three detectors Clearly the frequency of showers that span all our detectors $\leqslant 10^{-7}$ s^{-1} and so the probability of having three such showers within 3 ms $\leqslant 10^{-26}$ s^{-1} This process is also in disagreement with the small observed pulse heights

We have considered several other correlation chains including those involving local radioactivity, and have found similar low occurrence probabilities We are thus led to consider the 24 counts as correlated and probably due to ν_e interactions Using 24 counts in 12 m^3 of H_2O we obtain a lower limit ν_e to the $\bar{\nu}_e$ flux of 1 5 × 10^{11} (50 MeV/E_ν)$^2\bar{\nu}_e$/cm^2, or 1 2 × 10^7 erg cm^{-2} for 50 MeV anti neutrinos

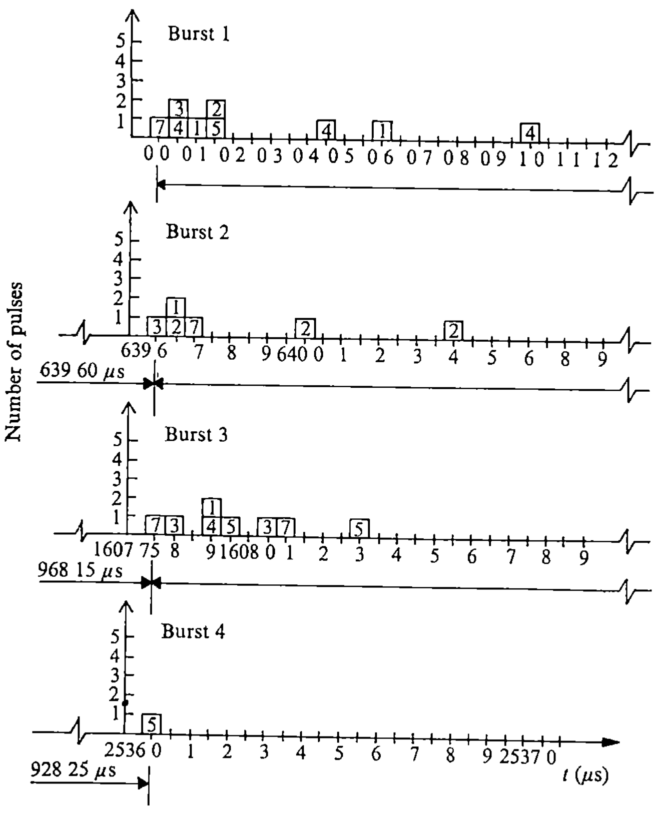

Fig 3 Time distribution of counter pulses within each burst The number within each box indicates the number of the counter giving rise to that pulse

No additional direct experimental information is available We can, however, speculate on possible sources such as the gravitational collapse of stars For such a source near the galactic centre ($d \approx 3 \times 10^{22}$ cm) we find a source flux of 1 4 × 10^{53} erg (for $E\bar{\nu}_e$ = 50 MeV) which is consistent with the prediction of Zel'dovich and Guseinov[5] for the emission of neutrino energy by a collapsing star Various people[6-10] have pointed out that a collapsed star is likely to 'bounce' a number of times and that the bouncing periodicity is ≈ 1 ms The narrowness of the pulses of emitted radiation may be a consequence of the fact that the collapsed star emits antineutrinos mainly during the period its density is maximum, at the minimum of its bounce

If we assume that the energy distribution of the $\bar{\nu}_e$ has a width comparable to $E\bar{\nu}_e$ then from the observed narrow pulse width we can place an upper limit on the mass of $\bar{\nu}_e$ of 10^{-2} eV/c^2

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Brookhaven solar neutrino detector and collapsing stars

THE Brookhaven solar neutrino detector has been in continuous operation in its present form since 1970 The basis of the detection method is the inverse beta decay reaction on ^{37}Cl to produce ^{37}Ar ($t_{1/2}$ = 35 1 d) which can be detected at a sensitivity level of 10 to 20 atoms The detector, consisting of 380,000 l of C_2Cl_4, is described in detail elsewhere (refs 1-3 and references therein) Measurements with this detection system establish[1-3] an upper limit for solar neutrinos of 1 SNU (1 SNU = 10^{-36} captures/^{37}Cl atom-s) This limit is in substantial disagreement

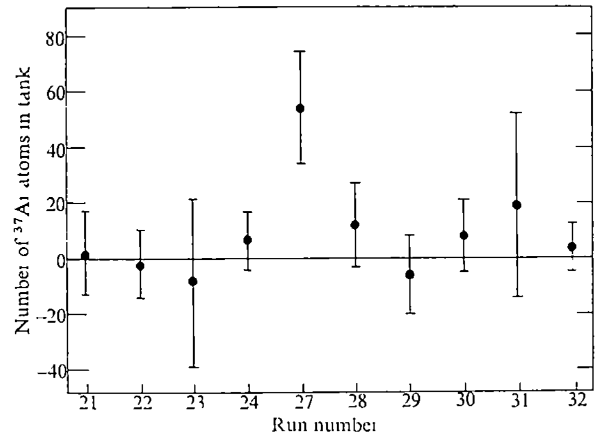

Fig 1 The number of ^{37}Ar atoms found in the C_2Cl_4 tank at the end of each helium sweep is plotted for each run A cosmic-ray background correction has been subtracted The error bars shown are purely illustrative and are based on the square roots of the small counted numbers

with accepted solar model calculations[4] which predict a result of 5 6 SNU (ref 5) The significance of this discrepancy has been recently analysed in detail[6], this number also represents a base line for comparison with other possible sources of high energy neutrinos[3,7], in particular those produced by collapsing stars

On January 4, 1974 a possible collapsing star event was observed by the University of Pennsylvania water Cerenkov antineutrino detector located next to the Brookhaven detector in the Homestake Gold Mine[8] The C_2Cl_4 tank was swept as soon as possible after this event in an attempt to detect an associated neutrino pulse The result of this search was negative and can be used to set a limit on the ratio of antineutrinos to neutrinos escaping from a collapsing star—if that is indeed the source of the event

Figure 1 shows the experimental results for ten runs with the Brookhaven neutrino detector in its present form The cosmic ray correction[9] amounts to a subtraction of about 3 to 4 atoms per run Of the ten measurements to date, only one, run 27 (July 7 to November 5, 1972) seems to contain any measurable ^{37}Ar, in contradiction with the other runs A simple statistical test, Chauvenet's criteria, suggests that the point is abnormal and for that reason it is not included in the solar neutrino calculation A more sophisticated statistical analysis of the data[10] also suggests that the measurement is anomalous The number of ^{37}Ar atoms in the tank at the time of purging run 27 (November 5, 1972) was 58 ± 20, compared to an average of 8 ± 7 ^{37}Ar atoms for all other runs The number of ^{37}Ar atoms in the tank produced by cosmic rays is estimated[9] to be 4 ± 2 Apart from statistical considerations, the data for run 27 show a rather convincing signature for ^{37}Ar Auger electron decays, agreeing well in energy, pulse rise time and half life The possibility that this ^{37}Ar was introduced by some type of contamination was carefully considered and found to be extremely unlikely The various contaminations considered were (1) the activity of ^{37}Ar in atmospheric argon from underground nuclear bomb testing and the amount of air argon introduced in the sample, (2) ^{37}Ar in the argon added directly to the counter filling, and (3) ^{37}Ar remaining in the counter and the filling line from previous measurements The amount of ^{37}Ar found was about 10 to 20 times the normal cosmic ray rate Pallister and Wolfendale[10] examined the possibility that this amount was simply due to a large fluctuation in the cosmic-ray interaction rate and concluded this was also extremely improbable A solar flare occurred on August 4, 1972 which was the largest ever observed But this is an unlikely source of neutrinos The experimental facility is sufficiently deep underground to be totally unaffected by the relatively low energy charged particle flux On September 2-11, 1972 a large radio outburst was recorded from Cygnus X-3, but again this is generally believed to be an unlikely source of neutrinos A γ-ray pulse of unusually sharp rise time was observed by the Vela satellites on November 1, 1972 (ref 11) The energy flux in this pulse was very small and it is difficult to say whether or not this event is related to the excess in run 27 The University of Pennsylvania detector was not yet operational at the time and so it is not possible to say whether or not the excess was related to a collapsing star

Run 32 was collected on January 25, 1974 about three weeks after the event detected by the University of Pennsylvania water Cerenkov detector The result, with cosmic-ray background subtracted, is 4 5 ± 9 ^{37}Ar atoms in the tank at the time of the sweep This is in good agreement with eight other runs which give an average of 4 ± 7 ^{37}Ar atoms (Fig 1) If a decay correction is included, a 1σ limit of $\leqslant$21 atoms of ^{37}Ar produced on January 4, 1974 is obtained To compare this number with the University of Pennsylvania result, a knowledge of the relative sensitivity of the two detectors is required The cross sections for the two reactions were calculated[12] using the best available values for the matrix elements and Fermi function A calculation of the ratio of the neutrino reaction (σ_{37}) to antineutrino reaction (σ_1) at various particle energies shows that the ratio is about 1 1 at 20 MeV (σ_{37} = 3 1 × 10^{-41} cm^2), the threshold for the Cerenkov detector, and approaches an asymptotic value of 1 6 at high energy At very high energies, the ratio would presumably start to drop again due to nucleon recoil effects, but that is outside of the energy range of interest for collapsing stars Since the chlorine detector contains about 2 6 times as many usable target atoms as the Cerenkov detector, the efficiency of the chlorine detector is 2 9 to 4 2 times that of the Cerenkov detector for equal particle fluxes and energies in the energy range of interest Combining this result with the 24 counts seen in the Cerenkov detector, a minimum of 70 atoms ^{37}Ar should have been produced in the chlorine detector for equal particle fluxes and energies The antineutrino to neutrino flux ratio is $\geqslant$4 1 assuming a typical particle energy of 50 MeV (σ_{37} = 2 9 × 10^{-40} cm^2) The pulse recording system used in the water Cerenkov system was only capable of recording a maximum of 24 pulses during the time period of interest There is therefore a large uncertainty in the true size of the event The corresponding flux ratio may in fact be significantly larger since the chlorine detector integrates the entire event

There are several restrictive conditions that must be satisfied if the January 4 event is to be interpreted as a collapsing star First, the expected time duration for a pulse of neutrinos to diffuse out of the central regions of a star is $\sim(R/c)$ (R/λ), where R is the characteristic size of the collapsing star ($\sim 10^{6\ 5}$ cm) and λ is the average mean free path of the antineutrinos In order for there to be significantly more antineutrinos than neutrinos leaking out, (R/λ) must be large The characteristic time for changes in the physical conditions is of the order of the dynamical time and is also long, ~ 1 ms So the main pulse width should be $>10^2$ μs instead of the observed width ~ 1 μs The main pulse should have (see equation (14) of ref 14) $\gtrsim(R/3)$ times (approximately 100 times) as many neutrinos and antineutrinos as in the prompt burst This increases the total estimate of energy in neutrinos and antineutrinos for the model of ref 12 to ~0 3 to $1M_\odot c^2$ × (distance/10 kpc)^2 if a fairly flat spectrum of energies is assumed

The failure to observe neutrinos associated with the event of January 4 is also a serious constraint on possible interpretations in terms of collapsing stars Under most astrophysical circumstances one would expect approximately equal numbers of neutrinos and antineutrinos whereas the combined observations imply an antineutrino flux more than four times the upper limit on the neutrino flux The University of Pennsylvania water Cerenkov detector is not sensitive to neutrinos below ~20 MeV whereas the Brookhaven ^{37}Cl detector has considerable sensitivity in this region[12] If one interprets the event of January 4 as being produced by antineutrinos then one must say the flux was primarily antineutrinos more energetic than

20 MeV But one numerical calculation of neutrino transport in a collapsing star yields[14] spectra with a peak at about 10 MeV

Also the initial stages of the collapse of a white dwarf to form a neutron star would produce predominently neutrinos[14] through electron capture, transforming protons into neutrons, and would yield very few antineutrinos (provided the star is made out of matter, not antimatter)

The likely production processes at higher temperatures and densities yield equal numbers of neutrinos and antineutrinos, this is true for all processes based on the interaction $e^+ + e^- \rightarrow \nu_e + \bar{\nu}_e$ and for two nucleon collisions ($n + n \rightarrow p + n + e^- + \bar{\nu}_e$, $p + n + e^- \rightarrow n + n + \nu_e$) In nondegenerate, high temperature regimes ($T^3_{20\mathrm{MeV}}\rho^{-1}{}_{\mathrm{nuclear}} \gtrsim 10^3$) such as might occur in shock waves formed by supernovae[15], the principal opacity would be due to lepton–lepton scattering[16] ($\nu_e + e^- \rightarrow \nu'_e + e^{-\prime}$, $\bar{\nu}_e + e^- \rightarrow \bar{\nu}'_e + e^{-\prime}$ and so on) which leads to equal opacities for neutrinos and antineutrinos if, as is true under such conditions, the number of electron–positron pairs in equilibrium with the radiation field is large compared to the number of atomic electrons (The maximum difference between ν–e and $\bar{\nu}$–e cross sections for any intermediate vector boson theory with μ–e symmetry is a factor of three[18] and for the Weinberg–Salam model with $\sin^2\theta_w = 0\,3$ to 0 4 is a factor of two) (compare ref 19)

Most astrophysical events are expected to give rise to at least as many neutrinos as antineutrinos Moreover, accompanying the prompt antineutrinos to which the University of Pennsylvania detector is sensitive one would expect a much larger number (on a longer time scale) of lower energy neutrinos (and antineutrinos) to which the Brookhaven detector is sensitive The absence of detectable neutrinos is puzzling if the event of January 4 was caused by antineutrinos from a collapsing star

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Possible explanations of the 'high' number of counts in the solar neutrino experiment

EVANS *et al*[1] (see also refs 2, 3) have presented data on the rate of production of ^{37}Ar with the Brookhaven Solar Neutrino Detector, two facts stand out the overall mean rate is low compared with expectation[4], and one run, No 27, may be significantly higher than the remainder The present note is primarily concerned with the statistical significance and explanation of this high rate The calculation of the rates and uncertainties is not a simple procedure and it is important to have an independent determination, with the cooperation of the authors, we have made our own analysis using their basic data (β-ray counter measurements of the β-activity of the abstracted ^{37}Ar over successive periods of ~30 d), for the important run 27

Several methods have been adopted to determine the rate R I, the maximum likelihood method, II, a similar method used in the Case-Wits-Irvine Experiment by Meyer[5], and III, a graphical method in which isoprobability contours of the observed configuration of counts are calculated, and cuts through them taken and the resulting, normalised differential probability curves integrated to determine the probability that the true number of atoms at the end of exposure was less than some particular value N_o

$$P(<N_0) = \int_0^{N_0}\int_0^{\infty} P(N_0,b)\mathrm{d}P\mathrm{d}b$$

where b is the small but finite counter background The values of N_0 at which $P(<N_0)$ are 16% and 84% correspond to the 'error' in the mean rate All the calculations gave R_{27} as 1 18 ^{37}Ar per day (some of the residual difference with the Davis estimate of 1 24±0 43 ^{37}Ar per day arises from small differences in input data) Methods I and II gave the standard deviation of the rate (σ=0 43), but because of the skew nature of the probability distribution this bears no simple relationship to the probability that the mean rate is within the above bounds Method III gave errors of −0 31 and +0 41 ^{37}Ar

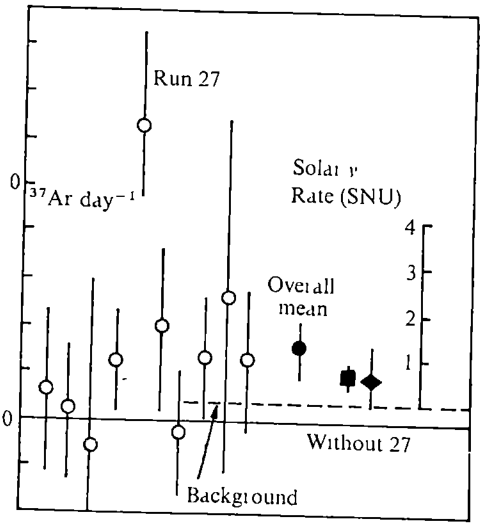

Fig 1 Results of the solar ν experiment, after Evans *et al*[1] The errors on individual run rates (open circles) quoted for Run 27 come from the present work, the rest from ref 1 The mean values are those derived by us

Table 1 χ^2 and probabilities for various situations

Adopted 'true' value	Data Examined	χ^2	$p(\chi^2)$
Overall	All data	11 7 (14 0, 21 8)	16% (8%, 0 5%)
mean	R_{27} excluded	2 9 (4 0, 11 0)	90% (78%, 14%)
Mean ex-	All data	13 2 (15 0, 22 2)	10% (6%, 0 5%)
cluding R_{27}	R_{27} excluded	1 8 (3 6, 10 7)	97% (82%, 15%)
Expected	All data	16 3 (18 8, 30 1)	4% (1 5%, 0 02%)
background	R_{27} excluded	2 6 (5 1, 16 3)	92% (66%, 2 3%)

The figures in parenthesis refer to the situation where the errors for runs other than No 27 are multiplied by factors of 0 7 and 0 4 respectively It is likely that the true situation gives χ^2 probabilities intermediate between the values in parenthesis

per day, an increase of 0 12 on the lower limit estimated by Davis, lending further support to the suggestion of the anomalous character of run 27

To establish the significance of the high value of R_{27}, various χ^2 tests have been performed using the data shown in Fig 2, including and excluding run 27, and using several reasonable estimates of the true mean rate The results shown in the table indicate that there is some evidence of an excess in R_{27} But as was pointed out by Evans *et al*[1], there is a suggestion that the quoted errors (other than R_{27}) are too large, this is borne out by the high probability (90%) for the appropriate conditions

An alternative approach is to examine the errors and the dispersion of the mean rates about their mean Excluding run 27, the dispersion error α, is 0 05 which is much smaller than the value expected ($\simeq\langle\sigma\rangle/\sqrt{n}\simeq0$ 12, where $\langle\sigma\rangle$ is the median of the individual errors), on the basis of the errors of the individual rates This confirms that the quoted errors are probably overestimates In view of the uncertainty of the individual errors, the χ^2 probabilities have also been calculated with the errors on runs other than 27 reduced by factors of 0 7 (the ratio of 'our' error on R_{27} and the quoted error) and 0 4 (the ratio of the dispersion and 'expected' errors) respectively The results (see the Table 1) show that the significance of the excess is considerably enhanced In particular, if the smallest errors are adopted then Run 27 is almost certainly anomalously high More data over the next year or so will surely enable a more definite conclusion to be arrived at

It is interesting that if the dispersion errors are correct, then, neglecting R_{27}, the upper limit on the residual solar neutrino rate is only 0 9 solar neutrino units (SNU), and the solar model calculations are in disarray[4] Inclusion of run 27 raises the upper limit to ~1 9 SNU and the discrepancies are not quite as severe

Assuming R_{27} is anomalously high, various possibilities arise

(i) The presence of an unusual, non-Poissonian, increase in background

(ii) The effect of a large solar flare (August 4, 1972)

(iii) The arrival of neutrinos from a cosmic source

The background is composed of two parts

(a) Cosmic ray neutrinos produced in the atmosphere contribute ~30% to the background and are extremely unlikely to have large fluctuations over the integrated time scale of the detector

(b) The contribution from protons, 'knocked on' by fast cosmic ray muons to the reaction ^{37}Cl (p,n)^{37}Ar Knowledge of the muon spectrum, nuclear cross section, and yield of ^{37}Ar atoms per interaction ($\propto$ (Energy transfer)$^{0\ 7}$, following Wolfendale *et al*[6]) enables the contribution to the background from rare events producing many ^{37}Ar atoms (when large fluctuations may occur) to be determined This contribution is very small which implies that the background fluctuation should be strictly Poissonian with a total mean value of 0 09±0 04 ^{37}Ar atoms per day

Solar flare. Evans *et al* have rightly implied that the muons formed from protons in the solar flare have insufficient energy to penetrate to tank depth More important is the increase in the neutrino flux from the low energy muons decaying high in the armosphere but their contribution ismuch too small to provide the excess in R_{27} (We thank C J Hatton for comments on this point)

Cosmic source. Evans *et al* have sought an explanation of the neutrino excess in terms of cosmic sources (the γ-ray pulse event of November 1, 1972)[7] The unusually short pulse width (~0 1s) is of the same order as the expected collapse time of a star to the neutron stage when a large neutrino burst is expected[8] The probability of the solar neutrino detector recording a signal of more than 50 counts per run from collapsing stars has been considered by us[9] and shown to lie in the range 10^{-1}–10^{-2} if all stars of mass >1 5 $M_\odot$ collapse Such a probability does not disagree with run 27 corresponding to a collapsing star (one ν-burst in 10 runs), and assuming R_{27} is anomalously high, we would regard this explanation as perhaps the most probable

Finally, regarding the exciting result reported by Lande[10] we point out that events of this type, if genuine, are detectable by extensive air shower arrays, when the positrons released in the detector simulate a shower of completely flat lateral distribution Comparing the threshold sensitivity of the two major arrays, 60 MeV m^{-3} μs^{-1} water at Haverah Park and 80 MeV m^{-3} μs^{-1}at Sydney, with the energy deposition observed by Lande (15–75 MeV m^{-3} μs^{-1}) both figures are seen to be in the region of observed energy deposition Neither array observed a coincidence with the 'Penn' event but this is understandable if the energy deposition was near 15 MeV m^{-3} μs^{-1}

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Anomalous mercury in near-bottom water of a Mid-Atlantic Rift valley

MERCURY analysis[1] of over 1,000 water samples from GEOSECS stations in the western Atlantic indicated extremely low mercury concentrations (2–40 ng l^{-1}) in much of the ocean between 15°N and 35°S, and identified a large core of water of relatively high concentration (80–400 ng l^{-1}) between 25°N and 50°N These increased concentrations may result[1] from the entrainment of Hg which diffuses from the active spreading centre of the Mid-Atlantic Ridge, by Mediterranean water which flows over

Table 1 Mercury in samples of seawater from the FAMOUS area

Sample number	Hg (ng l^{-1})	NaCl (‰)	F Cl (× 10^5)	O_2 (ml l^{-1})
2	1,380±90	35 024	6 66	—
10	1,190±90	35 020	6 64	6 11
11	1,420±90	35 012	6 66	6 10
13	1,330±90	35 031	6 69	6 04
15	1,280±90	35 014	6 70	—
16	980±90	34 966	6 64	—
17	900±90	34 985	6 69	6 09
28	1,180±90	35 017	6 70	5 91
29	1,000±90	35 033	6 62	—
59	920±90	35 018	6 74	5 94
61	900±90	35 015	6 70	5 92
66	1,030±90	35 014	6 64	6 01
67	870±90	35 014	6 70	6 00
68	1,130±90	35 012	6 76	6 03
69	880±90	35 012	6 74	5 99
Mean	1,090	35 012	6 69	6 01
s d of mean	±193	±0 017	±0 04	±0 07

the ridge into the western basin of the Atlantic Ocean Mercury concentrations are as high as 1,370 ng l^{-1} at a depth of 2,435 m at GEOSECS station 27 (42°00′N, 41°59′W), (ref 1)

Bostrom and Peterson[2] have described sediments enriched in Fe, Mn, B, As, Cd, V and Cr in areas of high heat flow on the East Pacific Rise They suggested that these sediments have incorporated precipitates of the volcanic emanations from active ridge crests White[3] examined the composition of hot spring waters and suggested that trace elements such as Ba, W, Zn, Hg and Cu could also be enriched in the products of outgassing Mercury in Japanese hot spring waters has been reported[5] to range from 100–30,000 ng l^{-1}

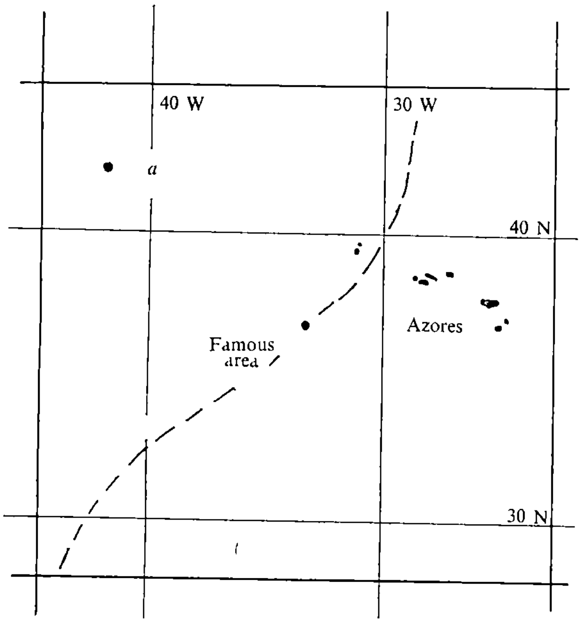

Fig 1 Location of the FAMOUS Area Dashed line, Mid-Atlantic Ridge, *a*, GEOSECS Station 27

During one phase of Operation FAMOUS (French–American Mid-Ocean Undersea Study), we took water samples from near the bottom of a rift valley at 36°49′N, 33°16′W (Fig 1), at a depth of 3,200 m The iron manganese ratio of the samples averaged 9 2, close to the average value of 10 observed for ocean water (unpublished data from this laboratory) The 15 samples were analysed for mercury using the method of Carr *et al* [5], with a best estimate of error ± 90 ng l^{-1} The analysis showed a mean value of 1,090 ng l^{-1}, with a standard deviation of the mean of 193 ng l^{-1} (Table 1) The difference between samples is significant All the samples seem to have come from the same water parcel, as is indicated by the uniformity of values for salinity, dissolved oxygen (Table 1), and by the temperature 3 97 ± 0 03° C The F Cl ratio was that of normal sea water These data support the hypothesis that mercury is introduced into the sea water column at active spreading centres

We wish to thank H Fleming, and the scientific complement and the officers and crew of the USNS Mizar for the opportunity to obtain water samples

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Three dimensional image reconstruction on an extended field—a fast, stable algorithm

WE describe an improved alogrithm (EFIRT) for solving iteratively the linear equations relating a three-dimensional density to a given set of its projections, when the unknown density is expressed as samples on a grid The need to solve such equations arises in electron microscopy, medical radiography, radio and X-ray astronomy and other fields The projection equations comprise a very large, sparse and often underdetermined set If the number of unknowns is not too large, the equations may be solved by a least squares procedure, using filtering to combat the ill-conditioning[1] The number of unknowns is often too great for such a procedure to be computationally feasible and iterative techniques[2,3] must be used Alternatively the Fourier transform provides a stable and computationally efficient means of solution[1] Sometimes use of the Fourier transform is not convenient, for example, if point rather than plane projections are given or if the projection data are weighted by a non-uniform but linear response of the measuring device Such problems are much more readily expressed in terms of projection equations and the development of stable methods for their solution is therefore important

If the observations contain noise the set of projection equations will in general be inconsistent This is aggravated by the approximations involved in representing the continuous density in the object by a discrete set of samples Existing iterative methods of solution are unstable in these circumstances, though the SIRT algorithm has proved much better than the ART algorithm[3,4] The Extended Field Iterative Reconstruction Technique (EFIRT) presented here attempts to overcome this instability by performing the reconstruction on an extended field which is larger than the object Unlike any iterative technique so far described, this allows errors and inconsistencies to propagate outside the object domain in the reconstruction The extended field effectively provides a 'rubbish bin' for a large proportion of the noise, so that it does not spoil the region of interest The rationale for this approach comes from a Fourier analysis of the problem

We take the simple case of data collected by tilts about a single axis, so that reconstruction reduces to solving for a two-dimensional section from a set of line projections We denote the unknown sampled density values in the object by

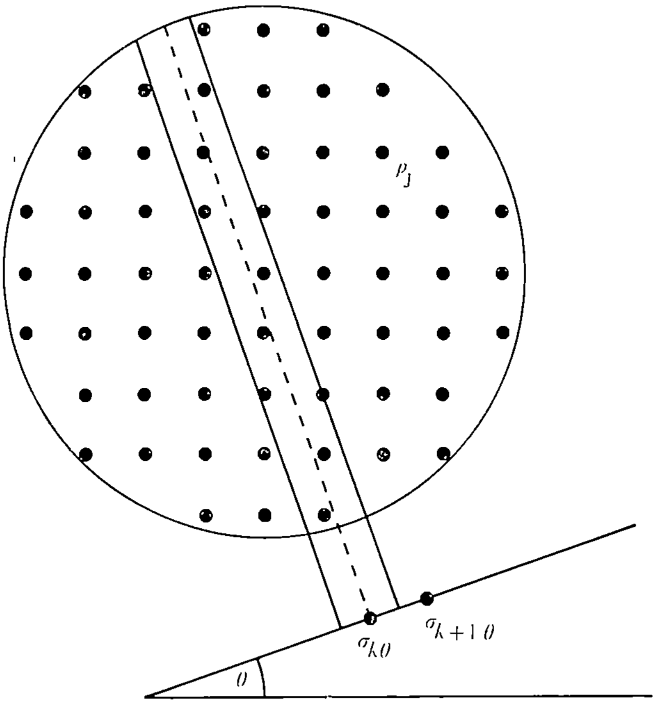

Fig 1 Densities ρ_j of the object are reconstructed from projection data $\sigma_{k\theta}$ The diagram shows the k^{th} ray for the projection at angle θ, which is used in calculating the ray sum $R_{k\theta}^{(q)}$ of the current approximation $\rho_j^{(q)}$ The number of sample points ρ_j included in the ray is $N_{k\theta}$ and the length of the ray within the circular object domain is $L_{k\theta}$ The width of the ray is equal to the spacing of the sampling lattice for the ρ_j

ρ_j and the total set of sampled projected densities by σ_i (Fig 1) The two sets of quantities are related by the projection equation

$$\sigma_i = \Sigma_j p_{ij}\, \rho_j$$

If a simple ray sum approximation is made, the elements p_{ij} of the projection matrix are 0 or 1 If some form of interpolation is used to express the density between sampled values ρ_j, the p_{ij} are fractions between 0 and 1 We assume the former simple approximation here

Initially all the density values $\rho_j^{(0)}$ are set equal to the mean density level in the object, which can be estimated from the projections We then generate successive approximations $\rho_j^{(q)}$ to the density using the formula[3]

$$\rho_j^{(q+1)} = \rho_j^{(q)} + (\sigma_{k\theta}/L_{k\theta} - R_{k\theta}^{(q)}/N_{k\theta}) \qquad (1)$$

Here $\sigma_{k\theta}$ represents the observed projected density at point k in the projection at angle θ, $R_{k\theta}^{(q)}$ represents the ray sum of the current approximation $\rho_j^{(q)}$ for the kth ray in the projection at angle θ, and $L_{k\theta}$, $N_{k\theta}$ are respectively the length of the ray lying in the object and the number of sample points ρ_j within it For a given j in equation (1), only one point k is included for each θ (Fig 1) It is used for all points in the first projection, then the second and so on until all projections have been used, completing one cycle of iteration Further cycles of iteration can then be made The use of equation (1) makes each ray sum $R_{k\theta}^{(q+1)}$ for the approximation $\rho_j^{(q+1)}$ equal to the observed projected density for the particular projection θ most recently used

The overall size of the object can be estimated from the given projections and we assume that it is contained within a circle of radius a If it is clear from the projections that the object is not isometric, some other choice of domain can be made The essential point of EFIRT is that we apply equation (1) not only to points within the object domain of radius a but to all points within a circle of radius b, say, where $b > a$ We thus have an annular region outside the object in which errors can propagate Initially the density $\rho_j^{(0)}$ is set to the mean density within the estimated object domain and to zero in the annular region In the iteration, those projected density values, $\sigma_{k\theta}$, which correspond to rays passing only through the annular region and not through the object, are taken to be zero

The density in the annular region arises from noise, inconsistencies and possibly 'fringe effects' due to the limited resolution obtainable from the finite set of projections and it need not therefore be positive Indeed, in general it will be equally positive and negative So the algorithm (1) must not be constrained to give positive density in the annular region, though the constraint can be applied within the object itself Clearly multiplicative algorithms, which correct each ray sum by multiplicative rather than additive scaling, are not applicable

With noisy data the use of this extended field leads to a great improvement in both the stability of the iteration and the quality of the resulting reconstruction From numerical experiments with a known test object and its projections, it is possible to tell how close the reconstruction is to the true object density after each iteration We express the root mean square difference in density between the reconstruction and the object as a fraction, δ, of the standard deviation of the density within the object Thus normalised, δ is a measure of how much closer to the object is the reconstruction, than would be a uniform distribution equal in value to the mean of the density in the object

The test object (Fig 2) consists of a set of uniform discs[3], whose projections are easy to compute analytically We have taken 25 equally spaced projections $(0 < \theta < \pi)$ The true projections are made noisy by adding at each point a pseudo-random number uniformly distributed in the interval $(-0\,1\bar{\sigma},\ 0\,1\sigma)$, where $\bar{\sigma}$ is the mean projected density In a second example each projected density is multiplied by a pseudo-random number from a Gaussian distribution with mean 1 0 and standard deviation 0 1 The course of the iteration is plotted in Fig 3 for the two examples We have held constant the radius a of the object at 12 sample steps and increased the radius b of the total field giving an increasing annular region for error propagation Shannon sampling corresponding to the Fourier cutoff $R_{max} = (25)/(\pi 2a)$ appropriate to 25 views[1] would give about 8 samples in the radius of the object, so the

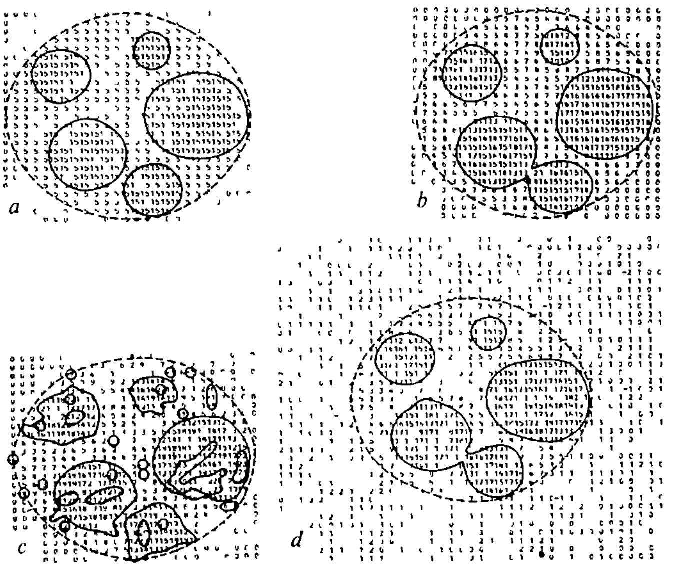

Fig 2 a, Model structure, consisting of a set of circular discs of uniform density, used for testing the algorithm The printout is compressed by 20% in the vertical direction giving a distorted appearance The reconstructions were made from 25 equally spaced projections to which 10% uniform noise has been added b, Reconstruction by R-weighted back-projection c, Reconstruction after 1 cycle of iteration with a non-extended field (b/a = 12/12) d, Reconstruction after 1 cycle of iteration with EFIRT (b/a = 24/12) Only part of the extended field is shown

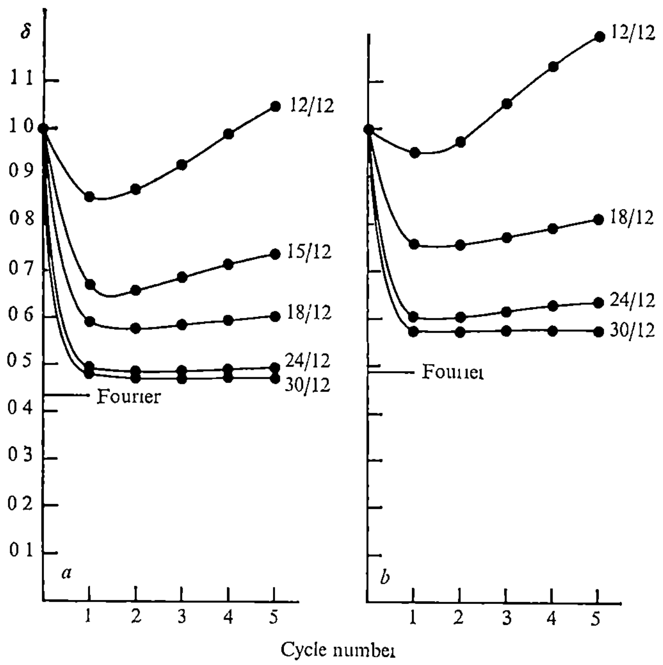

Fig. 3 The discrepancy δ (see text) plotted against the number of cycles of iteration of EFIRT, for the reconstruction of the object shown in Fig 2*a* from 25 projections containing noise The values of δ are computed using only those densities within the circle of radius *a* Each curve is for a different size of extended field as indicated by the value of *b*/*a* appended Also shown is the value of δ for a reconstruction made by a Fourier method, as described in the text In *a*, 10% uniform noise was added to the projections In *b*, 10% Gaussian multiplicative noise was included

sampling chosen is fairly generous Increasing the frequency of sampling to 18 in the radius produced only a slight improvement Presumably this is because the total number of grid points in the object domain is already about equal to the number of recoverable parameters set by the number of views[5]

When $b/a = 12/12$ and there is no annular region (so that we are essentially using the ART algorithm[2]), the value of δ falls slightly at the first iteration but thereafter increases rapidly giving rise to a reconstruction which bears little resemblance to the original The effect is of an increasing 'peppering' of the reconstruction (Fig 2*c*) But as the value of *b* is increased, the value of δ after the first cycle falls dramatically (Fig 3) Even a small extension of the field produces a significant effect Figure 2*d* shows the improved reconstruction obtained after one cycle when $b/a = 24/12$, again for the case of additive noise There is now density in the annular region outside the object Furthermore after the steep fall at the first cycle of iteration, δ tends to become more and more constant in successive cycles as *b* is increased (Fig 3) But increasing *b* beyond a certain size produces little extra improvement A similar behaviour was observed with other test objects although the effects were less marked when fewer views were used The extended field algorithm is so rapidly convergent that only one complete cycle of iteration is required, compared with about 15 cycles when using SIRT (ref 3) In spite of extra computation required when working with an extended field, the method is at least as fast as any other stable iterative method

Also indicated in Fig 3 are the values of δ obtained when the reconstruction is done by a Fourier transform method, in this case *R*-weighted back-projection[6,7] We perform one-dimensional transforms on the projections and weight[5,7] the frequency components by transform radius R out to a radius R_{max} and then by $(2R_{max}-R)$ out to a radius $2R_{max}$, where R_{max} is set by the number of views and the noise level We then inversely transform to produce a set of modified *R*-weighted projections The reconstruction is then made by back-projecting these modified projections The reconstruction from the data containing additive noise is shown in Fig 2*b* Although the values of δ obtained by this Fourier method are lower than the best δ achieved by EFIRT for both types of noise, the differences are probably not significant since the projection equations used in EFIRT were of the simplest type, without interpolation

Fourier analysis shows that besides the recoverable components giving density inside the object, there are a further set of recoverable components which build density outside the object[1,8] In the Fourier method[1], these external components are not explicitly calculated, but are implicit and serve to carry a certain part of the noise With perfect data from a spatially limited object, the external components will be zero and the set of projections can be completely interpreted, to a resolution set by the number of projections, in terms of the density within the object Once there is noise in the projections, the external components will be non-zero and the projections will no longer be completely interpretable in terms of density within the object In these circumstances, the projection equations set up for the object domain only, become inconsistent But by taking an extended field, which admits the possibility of building density outside the object, the equations become consistent and the convergence of the iterative procedure is stable and rapid Similar rapid and stable convergence was observed[2] when the ART algorithm was applied to pseudo projection data (when the ray sums $R_{k\theta}$ of the object are used in place of the true projected densities $\sigma_{k\theta}$), in which case the projection equations are again perfectly consistent, but this situation does not correspond to actual reconstruction from true projections

It is clear from Fig 3, that in these examples at least, most of the improvement to be gained by extending the field has already been obtained when the diameter of the total field is twice that of the object Further increases in the size of the field make only small changes to δ If all the external components were of equal weight, the annulus between *a* and 2*a* can be calculated from Fourier theory to contain about 75% of the external squared density It seems that in the examples considered here an even larger proportion of the total external density is contained in the annulus between *a* and 2*a* The optimal size of the extended field will almost certainly depend on the type of object being reconstructed and on the nature and amount of noise in the projections It is clear, however, that the extension of the field has a profound effect on the behaviour of the iterative procedure, and, contrary to what might have been expected, the prescription of the boundary of the object, far from being a useful constraint in solving the equations, is actually harmful

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Effects of sulphur content on superconductivity and intercalation in transition metal disulphides

THE nearly two-dimensional character of the group IVB and VB transition metal dichalcogenides intercalated with organic molecules[1] makes the superconducting metals logical choices for the study of superconductivity in two dimensions Many anisotropic semiconductors could be used as electronic devices But scientific investigation has been restricted partly by the quality of available crystals A number of complexes are metastable at room temperature, and others deintercalate below 150° C Many intercalation compounds become greatly exfoliated so that measurements of single crystals become quite difficult Other crystals exhibit little or no registry between adjacent layers In one case—TaS_2 (pyridine)$_{1/2}$—two phases of intercalated material have been detected We have found that the addition of sulphur to the intercalate both orders and thermally stabilises the resulting complex The removal of sulphur by the intercalate increases disorder and thermal instability

A layered, transition metal dichalcogenide can be intercalated with an organic molecule (typically a Lewis base) if the dichalcogenide and organic material are brought together at a sufficiently high temperature In the intercalation complex the organic molecule resides between layers of the dichalcogenide In each case where intercalation occurs the supernatant organic liquid changes colour In the case of ammonia intercalations in disulphides the colours range from dark blue to yellow and red For other intercalations in disulphides, such as pyridine and aniline, the colour is dark brown to black These colours have been found to be a result of sulphur in the supernatant Sulphur forms coloured solutions with ammonia[2] and reacts to form long chain polysulphides with amines[3] We have found that sulphur, dissolved in the various intercalates and heated to the intercalation temperature, forms a colour which is characteristic of intercalation at that temperature Comparison of the ultraviolet with the visible spectra has verified the presence of sulphur in the supernatant

Two experiments have been undertaken to determine the importance of the effect of sulphur on the intercalation product First, some crystals of TaS_2 were repeatedly intercalated with ammonia until the supernatant remained clear These crystals were then intercalated with pyridine Second, a number of crystals were intercalated from pyridine saturated with dissolved (molten) sulphur The results measured by the thermogravimetric analysis of deintercalation, are shown in Fig 1 From these data it is apparent that the TaS_2 (pyridine)$_{1/2}$ intercalated once from pure pyridine is a two-phase material, that preintercalation with ammonia produces the low temperature phase, and that intercalation with excess sulphur produces the high temperature phase

X-ray powder diffraction has shown that two layer spacings are associated with the two-phase material, 12 01 Å and 11 85 Å The phase that deintercalates at 100° C is a single-phase with $c=2\times 11\ 85$ Å and that which deintercalates at 300° C is single-phase with $c=2\times 12\ 01$ Å There is a qualitative difference between the diffractograms the 12 01 Å phase has very sharp lines characteristic of a highly ordered crystal, whereas the 11 85 Å phase has broad, rather diffuse lines, giving evidence of considerable disorder Thus, intercalation of pyridine from a saturated solution with sulphur produces a material with greater thermal stability, a larger c axis, less disorder, and little or no exfoliation

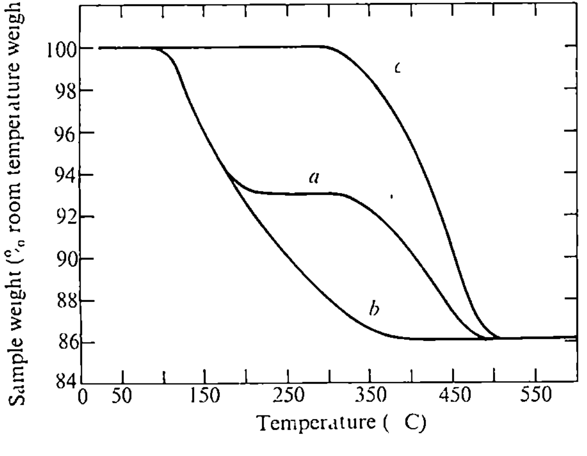

Fig. 1 Thermogravimetric analysis during deintercalation for three types of TaS_2 (pyridine)$_{1/2}$ *a*, intercalated from neat pyridine, *b*, preintercalated with ammonia—deintercalation is complete at 100° C, *c*, intercalated from a mixture of sulphur and pyridine—deintercalation occurs at 300° C

The addition or removal of sulphur also influences the electronic and magnetic properties of intercalation complexes Fragile, exfoliated crystals of TaS_2 (pyridine)$_{1/2}$ which are preintercalated with ammonia exhibit unusual anisotropies in the electrical properties at low temperatures[4] These same crystals have a superconducting transition temperature that is higher than that of crystals intercalated with excess sulphur We believe[5] that the rise in T_c in intercalated TaS_2 is related to the suppression of the low temperature crystal distortion and to non-stoichiometry on the metal rich side

Results which are essentially identical to these have been obtained from TaS_2 and TiS_2 intercalated with aniline Previously, only one phase had been observed in TaS_2 (aniline)$_{3/4}$ with a c axis parameter of 18 15 Å Intercalation from a sulphur-rich aniline solution produced an increase in c to 18 32 Å, with a corresponding increase in deintercalation temperature from 75° C to 150° C The increase of 0 17 Å in the c axis dimension is very similar to the value of 0 16 Å obtained with pyridine The sulphur-rich TaS_2 (aniline)$_{3/4}$ material also proved to be less exfoliated

Intercalation normally proceeds more slowly in the presence of excess sulphur, but in the case of TaS_2 (pyridine)$_{1/2}$ the intercalation time is doubled by the addition of sulphur The intercalation temperature seems to be correlated with the temperature at which sulphur reacts with the intercalate, thus, pyridine and sulphur do not react noticeably at room temperature, but form a dark brown-black, viscous liquid at 200° C This reaction has been attributed to the formation of polysulphide chains on the pyridine[3] A number of other intercalates have been tested (butylamine, N,N-dimethylaniline, and thiobenzamide) with similar results For example, butylamine intercalates in TaS_2 at room temperature and there it forms a black viscous liquid with excess sulphur

Only small amounts of sulphur are removed from the crystal during intercalation In several ammonia intercalations the amount was too small to weigh accurately and was certainly less than 1% of the total weight of the sample Sample intercalated in the presence of excess sulphur exhibit essentially the same increase in weight as materials intercalated with pure intercalate Thus, any mechanism involving the transport of one sulphur atom per intercalated molecule must be rejected

Hsu *et al*[6] have found that 4-vinyl pyridine can be intercalated in TaS_2, and that the thermal stability of the final product depends on the conditions of preparation They showed that the product obtained from preintercalation with aniline is less stable than the material prepared at 140° C from 4-vinyl pyridine and *p*-methoxyphenol (a polymerisation inhibitor) They claim that the increase in stability results from the *in situ* polymerisation of the 4-vinyl pyridine We find it more probable that they have produced two phases of the TaS_2 (4-vinyl pyridine)$_{1/4}$ complex, analogous to those reported here The addition of sulphur has the same effect on monomeric intercalations as does polymerisation they both increase the thermal stability

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Thin films from liquid crystals

THE properties of thin film drawn from liquids into air have been determined in many investigations[1–3], because the films form suitable systems for testing the theory of colloidal stability[4,5] Recent results have provided an improved understanding of the state of the aqueous layer of the film[6,7]

Films from liquids containing surfactants display stratification in the last stages of thinning[8], and the presence of a liquid crystal enhances foam stability[9] These facts have illustrated the structural connections between a thin film and the repeated lamellar unit in a liquid crystal[10] The lamellar liquid crystal itself has been used repeatedly as a model for the bimolecular layer of biological membranes The results[11] on the specific influence of the distance between layers in a lamellar liquid crystal on the diffusion coefficient of sodium and chloride ions, have further increased the interest in the structural relationship between lamellar liquid crystals and thin films with a bimolecular structure

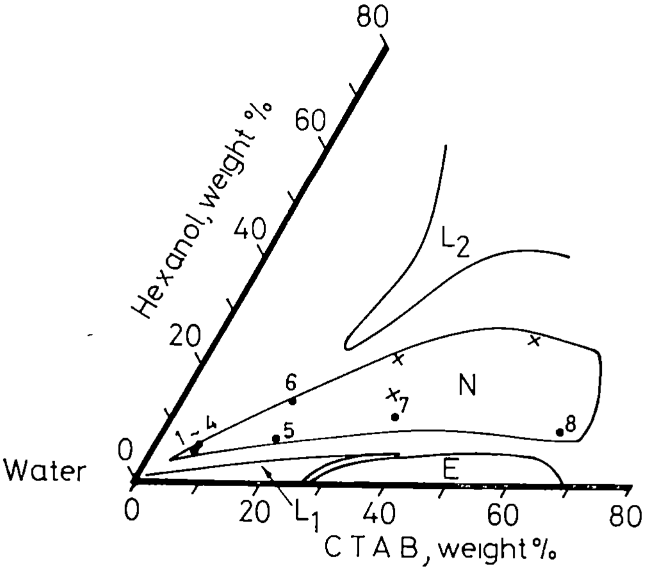

Fig 1 The composition of the liquid crystal samples (1–8) from which a thin film was drawn Crosses, compositions which did not give stable films, L_1, L_2, regions for isotropic liquid solutions, N, region of a 'neat phase' liquid crystal, E, region of 'middle phase' liquid crystal[12]

Previous investigations of these phenomena have, however, been concerned with comparisons between structure in thin films from liquids, and the dimensions in lamellar liquid crystals[10] We here report the first experiment in which a thin film has been drawn directly from a lamellar liquid crystal

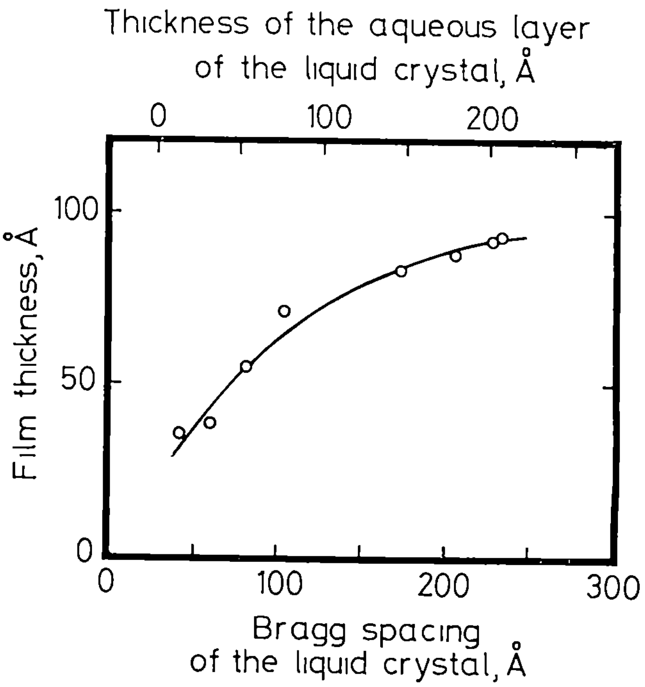

Fig 2 Thickness of the thin film against the dimension of the lamellar unit of the liquid crystal from which the film was drawn

A glass frame was used and the film was drawn slowly from the neat phase with known spacings[12] in the system water, cetyl trimethylammonium bromide (CTAB) and hexanol (Fig 1) The thickness of the film did not decrease gradually like a film from a liquid, but reached the black film state discontinuously from large thicknesses Consequently, in the calculation of thickness the Rayleigh equation had to be applied directly after determination of the intensity of the laser beam The results will be less accurate than those obtained using a combination of the intensity from the silvery and the black film The com-

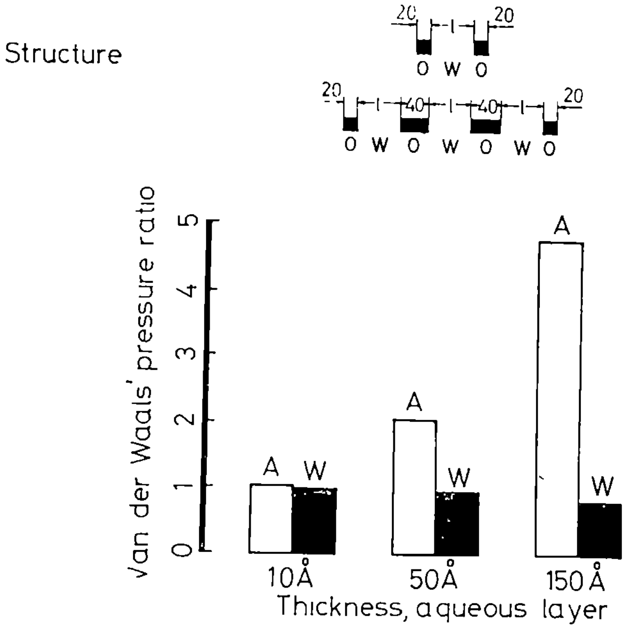

Fig 3 The ratio between structures I and II of the Van der Waals pressure over the central aqueous layer W (upper part of the figure), water layers, the thickness of which was varied according to the lower part of the diagram White columns denoted by A imply infinite layers of air outside the outer oil layers, black columns represent corresponding water layers

parison of the value from the two methods for a thin film from a solution gave, however, a difference of less than 3% The thickness was corrected according to Frankel and Mysels[13]

The results show that the film was thinner than the corresponding distance in the liquid crystal This difference became more marked as the thickness of the lamellar unit increased (Fig 2) One reason for this behaviour was found in the change in Van der Waals pressure when the adjacent layers in the liquid crystal were replaced by air to give the thin film The pressure over the central aqueous layer was calculated using the Lifshitz approach[14] with the extensions and approximations of Parsegian[15] Increase of the Van der Waals pressure over the central layer occurred when three bilayers were reduced to one, pressure increased rapidly with the thickness of the layer (Fig 3) The pressure change when the number of layers was increased from three to higher numbers was negligible

A thin film in water gives a slight decrease of the Van der Waals pressures in the last stages of thinning (Fig 3) and the liquid crystal in the water did not thin

Our results show that the liquid crystal may well serve as a good model for a thin film surrounded by water, in air the usefulness seems to be limited to films which have a central aqueous layer less than 50 Å thick The use of a lamellar liquid crystal as a model to clarify the structure of biological membranes thus seems justified

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Excess ^{26}Mg in the Allende Meteorite

VARIATIONS in the isotopic abundance of magnesium (78 6% ^{24}Mg, 10 1% ^{25}Mg, 11 3% ^{26}Mg) have been sought[1,2] in meteorites because of the possible addition of radiogenic ^{26}Mg by the decay of ^{26}Al This short-lived nuclide (half-life 7 4×10^5 yr) is formed by proton bombardment of light elements[3] and is of particular interest in theories of nucleosynthesis and heat generation during the early history of the Solar System Analysed material must have a high Al/Mg ratio to avoid the dilution of any radiogenic ^{26}Mg by common Mg Consequently, previous workers have chosen mainly feldspars from basaltic achondrites and metamorphosed chondrites The apparent absence of excess ^{26}Mg in such meteorites is, however, not surprising, because the condensation of chondritic material from the solar nebula now seems[4] to predate its later metamorphism by some 80 Myr, and the magmatic activity that produced the basaltic achondrites is younger still Thus, the feldspars that formed as a result of these processes would incorporate aluminium that was separated from a nucleo-synthetic event by at least 80 Myr and because of its short halflife insufficient ^{26}Al would remain to generate a detectable ^{26}Mg anomaly

A new possibility for the generation of magnesium isotope anomalies has arisen from the discovery by Clayton *et al*[5] of excess ^{16}O in the Allende Meteorite, and from

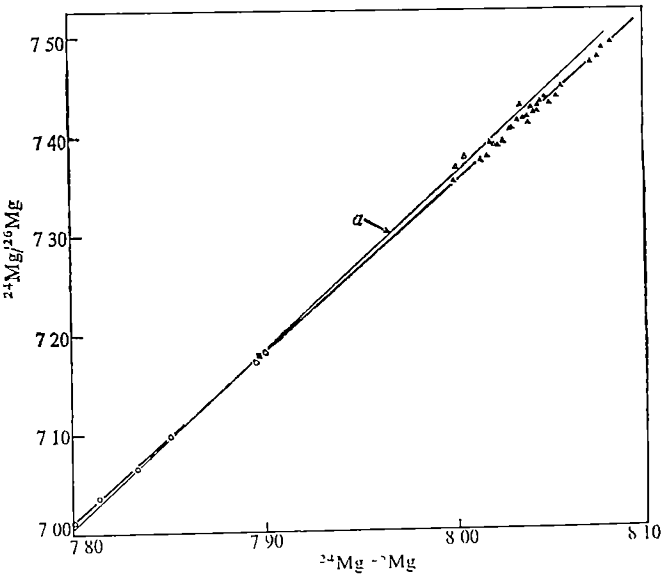

Fig. 1 ^{24}Mg/^{26}Mg against ^{24}Mg/^{25}Mg as measured by silica-gel[2] (open triangle) and triple-filament[7] (open circle) runs for the same terrestrial pyroxene, the runs were chosen to show extreme isotopic fractionation *a*, One stage Rayleigh fractionation, solid square, absolute value The mass spectrometer was a single focusing, 23 cm, 60° sector machine designed for high ion transmission[10], with magnetic field switching, Faraday cup collection and a calibrated digital readout for the electrometer Peaks other than ^{23}Na and ^{27}Al were never observed in scans of up to 10 mass units above and below ^{24}Mg, so that spectral interference from hydrocarbons and doubly charged ions can be discounted Data were collected when the ^{24}Mg beam current was within the range 5×10^{-12} and 5×10^{-11} A Each point in both Figs 1 and 2 comprises the mean of a set of fifteen ^{24}Mg/^{26}Mg ratios and the combined mean of one preceding and one following set of fifteen ^{24}Mg/^{25}Mg ratios The data are dispersed because of isotopic fractionation produced by diffusion and evaporation during the ionisation processes in the mass spectrometer Only silica-gel runs were made with the meteoritic samples, as this mode of ionisation is far more sensitive Sample sizes were of the order 1×10^{-6} g Mg, and laboratory contamination was approximately 7×10^{-9} g, mainly from the silica gel, which caused a maximum correction of 0 03% to two runs on chondrule 1 Magnesium was separated from the samples by standard silicate dissolution procedures followed by cation exchange in HCl

their prediction of possible excess ^{24}Mg derived from stellar nucleosynthesis

The Ca–Al-rich chondrules in the Allende Meteorite are the oldest objects known[4] They are comparable chemically to the initial condensates from the solar nebula[6], and their age, chemistry (high Al/Mg ratios) and unmetamorphosed character make them promising candidates in the search for radiogenic ^{26}Mg

Variations in the isotopic composition of magnesium may result from mass-dependent fractionation in physical and chemical processes, and from nuclear effects Mass-dependent fractionation is unknown in nature, but it occurs in the mass spectrometer used for isotopic ratio measurement That, and the variation with time during mass analysis, make the determination of absolute isotopic ratios exceedingly difficult The changes in the various magnesium isotopic ratios observed during measurement are, however, correlated with each other, because of the essential mass-dependence of the isotopic fractionation process Measured isotopic ratios from runs on a terrestrial pyroxene illustrate the effect (Fig 1) All points fit the same line even though two different methods of ionisation were used, and 'absolute' magnesium isotopic ratios[7] also fit It is curious that the slope of the line, 1 695±0 013 (σ), from the pooled data of all runs including those on meteorite samples, is less than the calculated slope for the single stage Rayleigh distillation (1 79 for Mg) that is usually fitted by the isotopes of other elements That can be attributed, however, to the operation of a multistage fractionation process A sample with an excess of one or more magnesium isotopes because of a nuclear effect would also define a line of the same gradient, but it would be displaced from the terrestrial line Thus, a nuclear isotopic anomaly can be detected despite the effects of variable isotopic fractionation during mass analysis Furthermore, a numerical correction for fractionation can be made by adopting an arbitrary standard value for one of the magnesium isotopic ratios, for instance, ^{24}Mg/^{25}Mg, and calculating the so-called 'normalised' value for ^{24}Mg/^{26}Mg by translation along the fractionation line In the case of magnesium it is essential to use the empirical slope, 1 695

We have analysed a melilite-bearing chondrule[8] (1) comprising large melilite crystals in a matrix of very finely crystalline grossularite, an aggregate[8] (2) containing spinel and enstatite, and an aggregate[8] (3) containing spinel and diopside It was not possible to remove all of the adhering matrix from the last sample, and the analysed material includes some foreign magnesium

Minerals were identified using X-ray powder diffraction Interpretation of the photographs was, however, difficult and unrecognised phases are doubtless present Chemical analyses by electron microprobe are given in Table 1

Table 1 Chemical composition of Allende inclusions

	Inclusion 1 Melilite	Inclusion 1 Bulk*	Inclusion 2 Bulk*	Inclusion 3 Bulk*
SiO_2	25 4	29 6	29 2	36 4
TiO_2		1 6	0 5	1 1
Al_2O_3	29 9	35 2	35 5	21 5
FeO		2 4	2 1	12 0
MgO	2 4	2 4	13 0	11 2
CaO	42 3	27 1	18 5	15 0
Na_2O		1 5	1 3	2 7
K_2O		0 1	0 1	0 1
Total	100 0	99 9	100 1	100 0
$(Al/Mg)_{atomic}$	9 8	11 6	2 2	1 5

* Electron microprobe analysis of a fused 2 mg sample

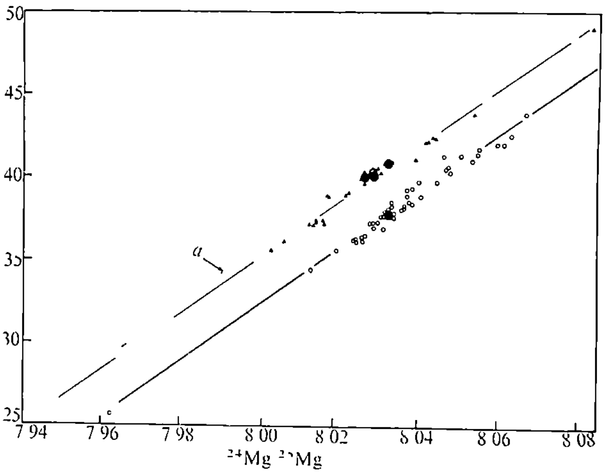

Fig 2 ^{24}Mg/^{26}Mg against ^{24}Mg/^{25}Mg for the four runs (Table 2) of the anomalous chondrule 1 (open circles) compared with the three runs on Px 5415 (open triangles) *a*, One stage fractionation, solid circles, average for 'unfractionated' runs The points for chondrule 1 define a separate fractionation line lower than the pyroxene line in ^{24}Mg/^{26}Mg but with the same mean ^{24}Mg/^{25}Mg All other data in Table 2 fit the pyroxene line

All but one of the analysed samples has a terrestrial isotopic composition The anomaly in chondrule 1 (Table 1) can be displayed on a plot of measured isotopic ratios (Fig 2) which compares data for the chondrule with those from a terrestrial sample The data for the chondrule fit a fractionation line that has a slope identical to the terrestrial line (respectively, 1 70±0 04 and 1 68±0 02) but is displaced from it towards lower ^{24}Mg/^{26}Mg Thus we have detected a nuclear effect in the magnesium isotopic composition of chondrule 1

Five out of fifteen runs showed no variable isotopic fractionation They are termed 'unfractionated' in Fig 2 although they are clearly biased with respect to the Catanzaro[7] 'absolute' values For these runs, as well as for the means of the fractionated runs, the ratios ^{24}Mg/^{25}Mg are all nearly the same This fact strongly implies that the principal component of the nuclear effect in Fig 2 is undoubtedly an excess of ^{26}Mg The entire effect may result from the addition of a pure ^{26}Mg component to common magnesium Our data do not, however, exclude the possibility that very small amounts of ^{25}Mg and/or ^{24}Mg may be present in the added ^{26}Mg-rich component as well The ^{24}Mg/^{25}Mg ratio of this hypothetical magnesium could be greater or less than in terrestrial magnesium

Because any difference between the ^{24}Mg/^{25}Mg ratios of chondrule 1 and common magnesium is so small, the results can be described by normalising values of the ^{24}Mg/^{26}Mg ratio to a fixed ^{24}Mg/^{25}Mg value For that we have chosen our mean measured ^{24}Mg/^{25}Mg (8 03) rather than the 'absolute' value thus minimising the shifts which result from normalisation and also any error resulting from uncertainty in the slope of the fractionation line (Table 2) The terrestrial isotopic composition was determined in two pyroxenes, six runs giving a mean ^{24}Mg/^{26}Mg ratio of 7 404±1 The most precise of the runs seems to be an outlier (Px 5414 R2) but there are no grounds for its rejection Measurements on the total Allende Meteorite fall into the range of the terrestrial population (mean 7 406±1) as does that for aggregate 2 (7 406±2) The mean ratio for aggregate 3 (7 402±1) is slightly lower than the terrestrial value and may be anomalous, although it is matched by the outlying terrestrial measurement Two separate dissolutions of chondrule 1 were both analysed twice and the results agreed excellently The mean value of (7 374±1) is quite distinct from the terrestrial range, indicating a 0 41% anomaly

There are very obvious needs to find anomalous magnesium in other Allende inclusions, to find the particular mineral phase(s) which bears the anomaly, and to relate the magnesium anomaly to the excess ^{16}O found by Clayton *et al* [5] Nevertheless, some conclusions can still be drawn First, because of the constant ^{24}Mg/^{25}Mg ratio, it is likely that the magnesium anomaly of chondrule 1 results entirely from excess ^{26}Mg, and this in turn implies strongly an origin by the radioactive decay of ^{26}Al formed during intense proton irradiation in the early stages of the Solar System As the ^{26}Al must have been made within the Solar System because of its short halflife, then it is likely that the excess ^{16}O in the Allende Meteorite and also other short-lived nuclides such as ^{129}I (ref 9) were formed within the Solar System, rather than outside it[5] Second, the absence of a comparable magnesium anomaly in the other two chondrules can be attributed easily to their law Al/Mg ratio (Table 1) This strengthens the idea that the anomaly results entirely from radiogenic ^{26}Mg On the basis of a 0 4% excess of ^{26}Mg for chondrule 1, excesses may be expected of 0 08% and 0 05% for aggregates 2 and 3, respectively, from their Al/Mg ratio, of which the 0 8% excess may just be detectable (compared with 0 035% for the coefficient of variation of the six pyroxene runs) If the failure to detect an anomaly in aggregate 2 is not because of insufficient data, two other possibilities may be suggested the introduction of common magnesium after the assembly of the Allende Meteorite (alkalis have been added to many such chondrules, particularly to aggregates, after more than 10^9 yr[4]), or the condensation of much of of the material of inclusion 2 at a later time than that of chondrule 1 Finally, we note the value $3\ 8 \times 10^{-5}$ that may be calculated for the ^{26}Al, ratio in condrule 1 at its time of chemical isolation That may be related to the radioactive heating of planetesimals[2] and provided that accretion occurred immediately after the formation of ^{26}Al, it is more than sufficient to account for the heating to metamorphic temperatures of chondritic planetesimals

Table 2 Normalised ^{24}Mg/^{26}Mg ratios*

Sample	Dissolution	Run	Number of ratios	Mean ^{24}Mg/^{26}Mg
Terrestrial pyroxene				
Px 5415	Mg4	R2	300	7 400±1†
		R4	180	7 404±3
		R5	480	7 404±2
Px 5641	Mg3	R1‡	195	7 405±2
		R2	240	7 404±2
		R3	225	7 407±2
Allende Meteorite				
Total meteorite	Mg1	R2‡	240	7 406±2
		R3‡	225	7 407±3
Inclusion 1	Mg1	R1	180	7 375±3
		R2	105	7 372±3
	Mg2	R1	345	7 374±1
		R3‡	270	7 374±2
Inclusion 2	Mg1	R1	135	7 406±2
Inclusion 3	Mg1	R1	345	7 401±1
	Mg2	R1‡	330	7 402±1

* ^{24}Mg/^{25}Mg = 8 03

† Uncertainty is the 95% confidence limits for the mean and refers to the last digit

‡ Runs with no variable isotopic fractionation

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Magnetic telechemistry is elegant but nature is complex

VOGT and Johnson[1] have suggested that the bulk chemistry of *in situ* abyssal basalts may be diagnosed using sealevel magnetic anomalies Their suggestion is based on two sets of assumptions and observations

First, they assume that the concept of mantle 'plumes'[2,3] is a valid way of explaining the existence of selected oceanic island platforms and other positive elements on the sea floor, and that 'plumes' are associated with increased magnetic anomally amplitudes

The second assumption is that the observed increases of magnetic anomalies which are associated with the Galapagos positive element, or 'plume', result from a relative increase in Fe and Ti concentrations The assumption is based on a comparison between published chemical data and anomaly amplitudes for the Juan de Fuca and Gorda ridges

In contrast to Vogt and Johnson's assertion, however, oceanic platforms (the most idealised surface expression of 'mantle plumes'), or 'hot spots'[1,5], do not generally have magnetic anomalies of significantly higher amplitude A characteristic of most island platforms is the general loss of the high amplitude linear magnetic anomalies which are normally caused by dyke injection during seafloor spreading[6,7] This is probably because the extrusive igneous activity increases relative to intrusive activity during the formation of the island platform, the process also involves hydro-explosions[8] as the platform becomes shallower than 500 m (ref 9) A probable concurrent loss of a regular stress field also occurs, because of the mass of the platform on the seafloor[10] Vogt and Johnson recognise that problem they comment that magnetic anomalies above the Cocos Ridge and Carnegie Ridge, associated with the Galapagos 'Plume' are low amplitude So they invoke the local existence of non-magnetic layers of tephra, and layers with opposing (cancelling?) polarities Similarly, Campsie *et al* [3] have suggested that these ridges may be chemically composite partly 'plume' and partly normal oceanic ridge materials These special arguments detract

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severely from Vogt and Johnson's idea Their high anomaly–mantle plume association is based on high and low magnetic anomalies observed over the Juan de Fuca and Gorda ridges, respectively, which they claim are 'plume' and 'non-plume' material, respectively An equally feasible explanation is that the standard deviation of the width of the zones of active dyke injection[6 7 11] is narrower for the Juan de Fuca Ridge than it is for the Gorda Ridge

Although there is a direct relationship between the FeO content (%) of dredged samples and the amplitudes of the magnetic anomalies for the Juan de Fuca Ridges (ref 1 Fig 2), there is no increase in the amplitudes of the anomalies over the Iceland 'plume' despite the high Fe and Ti content in the dredged rocks The absence of expected correlation is attributed to an 'anomalous' situation "increasing ash content and lava fracturing at low hydrostatic pressures" This does not, however, prevent Vogt and Johnson from using chemical data from the Rekjanes Ridge[12] in order to extend their idea to a possible inference of several different elemental concentrations

Although Vogt and Johnson clearly state their assumptions, much evidence exists to show that a constant Fe/Ti ratio could not be maintained reasonably during an unequal increase in the total concentrations of FeO and TiO_2 in basalt Variation of the intensity of magnetisation, *J*, within the $FeO–Fe_2O_3–TiO_2$ system[13], shows that factors such as oxygen fugacity, degree of quenching, and oxyexsolution, are much more important controls of *J* than simple FeO and TiO_2 concentrations[14 15], unless order of magnitude differences are involved Of major importance is the fact that the Curie point decreases to below room temperature with increasing TiO_2 content and decreasing oxidation state of Fe, thus rendering as minimal the prospects of high remanent *J* in such rocks Magnetic stability (which depends primarily on magnetic grain size[14,16]) is also important if it is absent, then no reversed polarity would persist and the essential polarity variation required for the production of high amplitude anomalies would not occur Unless of single domain size, Fe is quite unstable, and so an increase of Fe alone would not necessarily contribute to an increase in anomaly amplitude For Johnson and Vogt's proposal to be correct, it would (as they state) be necessary for 'plumes' to generate greater amounts of titanomagnetite, with relatively unvarying Fe/Ti ratios, and grain sizes But these parameters are to a large extent a function of the cooling rates of the igneous bodies involved, so it seems very unlikely that they would remain constant between those bodies which constitute abyssal crust and oceanic platforms, which have inherently different modes of emplacement Different modes of igneous emplacement alone can vary drastically the amplitude of magnetic anomalies[10], and as primary chemical properties do not depend on the mode of emplacement, there is no reason whatsoever to believe that they are the controlling factors in the character of all magnetic anomalies

Despite the elegance of Vogt and Johnson's idea, I suggest that insufficient rigour had been applied to the concept of magnetic telechemistry

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DRS VOGT AND JOHNSON REPLY We re-emphasise that we never attributed all of, or even most of, the amplitude variations of oceanic magnetic anomalies to the chemistry of basalt We simply proposed that chemistry does play a role in certain areas and should not in any case be ignored as a possible factor Even if the hypothesis explains only three areas of high amplitude anomalies[1] this amounts to about 8×10^5 km^2 of oceanic crust, certainly more than a very restricted marine geological circumstance

At the outset Watkins wrongly asserts that 'magnetic telechemistry' is based on the acceptance of 'mantle plumes' But the connection between high magnetic anomalies and high Fe–Ti concentration never depends on whether the geochemical anomaly is the result of differentiation in the uppermost mantle[2] or on whether there are two distinctive 'primary' (Fe–Ti rich) and 'depleted' magmas in the mantle[3] The hypothesis does not depend on the fixed position of the hot spots with respect to the Earth's spin, nor on whether there is deep rising convective motion below the hot spots[4] But areas of high magnetic anomaly are found in association with some hot spots, and so the two phenomena are apparently related

Hot spots can be 'Icelandic' (close to spreading axes) or 'Hawaiian'[5], only Icelandic hot spots can have a 'telechemical' signature The topographically positive element of Icelandic hot spots really consists of at least two parts First, there is an aseismic ridge or platform, or volcano chain, distinctly, and relatively sharply, elevated above the surrounding oceanic crust Examples are the Cocos, Carnegie, and Iceland–Faeroe ridges and the seamount chains extending north-westwards from the Juan de Fuca Ridge These ridges represent, in the stationary hot spot hypothesis, trajectories of plates over the deeper mantle Such ridges are constructed at least in part by 'Hawaiian' igneous activity extrusive and intrusive activity away from the spreading axis If some sort of spreading is involved in their creation, it is of a more complicated variety Surely this is so on Iceland These ridges usually involve an oceanic crust thicker than normal Perhaps as a result of these differences, many aseismic ridges have confused magnetic signatures of relatively lower magnetic amplitude There are obviously factors other than basalt composition involved, and there was never any reason to expect that an unqualified telechemical hypothesis could explain the magnetic signatures of the Cocos, Carnegie, Iceland–Faeroe, and similar ridges or platforms These aseismic ridges are simply another and more complicated form of oceanic crust, but are distinct as far as magnetic anomalies are concerned

The second part of the topographic anomaly is a broad, smooth, regional elevation in the spreading axis and older isochrons It is here that we observe, at least in some cases, regions of unusually high magnetic anomalies of seafloor spreading origin Parts of these regional bulges are also characterised by relatively smooth basement and reduced seismicity, but whereas the aseismic ridge *sensu strictu* is sharply demarcated, this broader bulge is not In the Iceland area the

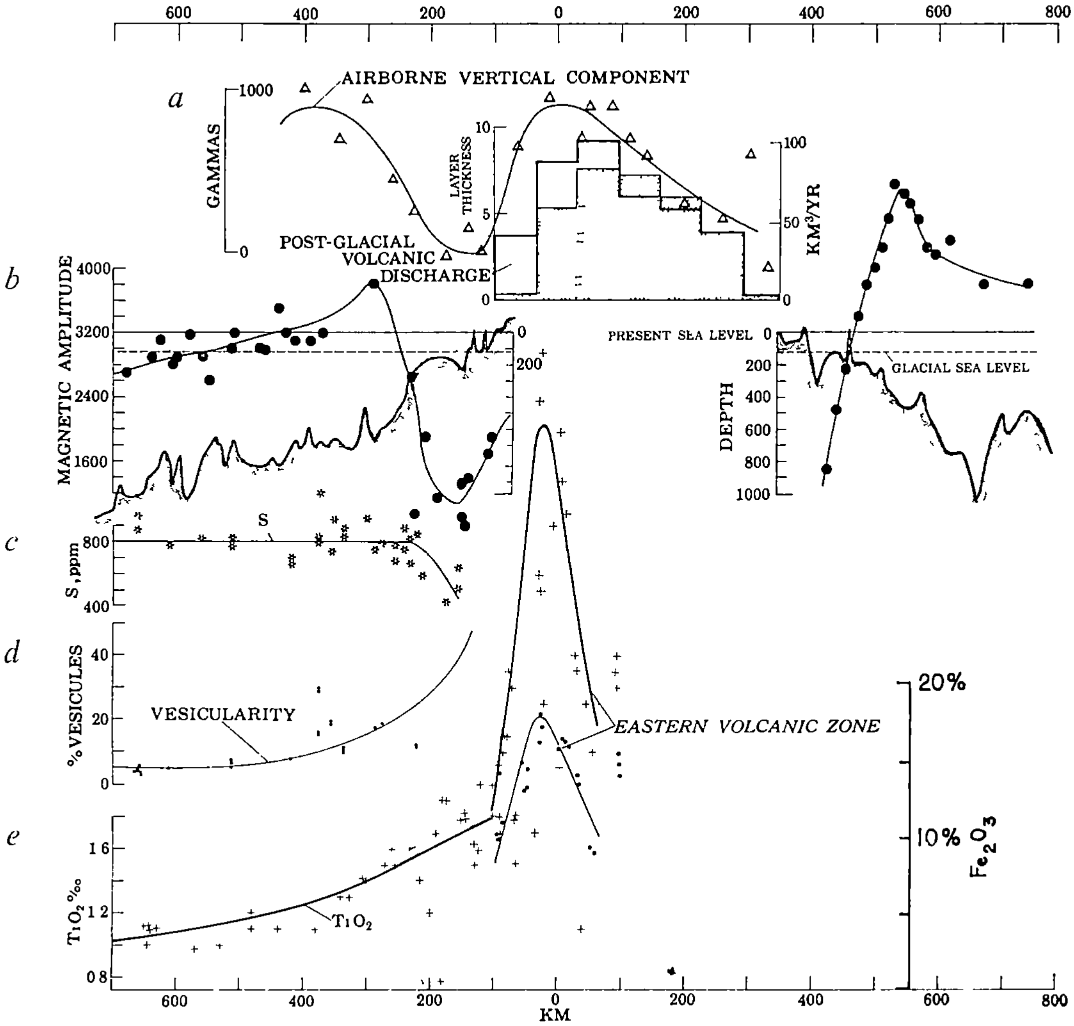

Fig 1 Chemical and physical parameters plotted against distance from the centre of the Iceland plume along the axis of spreading North-east to the right *a* Triangles, maximum vertical component[10] magnetic anomaly relief (axial high to negative west of it along Reykjanes Ridge), heavy line histogram, postglacial volcanic discharge (km^3 yr^{-1}), (ref 8) *b*, Axial magnetic high of the western negative (from the shipborne profiles[11,15], *c*, sulphur content[6], *d*, vesicularity[6], *e* Fe_2O_3 (filled circles), and TiO_2 (crosses) along the Reykjanes Ridge[3] and eastern neovolcanic zone of Iceland[8] Bathymetric profile along the axis was constructed from United States Naval Oceanographic Office charts Note the approximate correspondence in peaks of TiO_2, Fe_2O_3, discharge, and the maximum vertical component of the magnetic anomaly Where these quantities peak may mark the plume centre There is a sharp drop of anomaly amplitude as the spreading axis rises to within less than about 400 m of sea level This may reflect increasing oxidation states of titanomagnetites as volatiles are lost at low pressures[16], as evidenced by the high vesicularity[7] and low sulphur[7] in the same region Between 300 and 700 km south-west and 550 to 750 north-east the systematic variation in the anomalies is primarily the result of varying source depth

regional bulge extends southward through the Reykjanes Ridge to the Gibbs Fracture Zone, some 2,000 km from the centre of Iceland, it seems to extend a similar distance northwards The Greenland–Iceland–Faeroe Ridge, although concentric with the broader bulge, is an order of magnitude narrower Similarly, the Cocos and Carnegie ridges are only 50–100 km wide whereas the topographic bulge of the Galapagos Ridge, centred north of the Galapagos Islands, extends 500–600 km westwards, and eastwards and is up to 500 km wide at the longitude of the islands The Juan de Fuca Ridge is 500 km long, much more extensive than the width of seamount chain(s) terminating near its axis

The Iceland area (Fig 1) is, Watkins claims, another 'exception' to our hypothesis In fact, the Mid-Oceanic Ridge rises above sea level there, and there was no *a priori* reason to expect magnetic anomalies not to be affected It has been known for a long time that the magnetisation of subaerial and suboceanic basalts is quite different, even if initial magma compositions were identical Beginning at depths of 500 m or so, and increasing at shallower depths, vesicularity and explosiveness of the lavas increase as they erupt under lower confining pressures The greater fracturing and ash content would decrease the bulk magnetisation of the oceanic crust[1], degassing at vesicularities above 15% (~500 m depths) results in reduced sulphur concentrations[6] High *J* values of oceanic pillow basalt are possible because rapid quenching allows the retention of volatiles and the maintenance of titanomagnetites in low oxidation states[7] As the Reykjanes Ridge shoals toward sea level, volatiles are lost (Fig 1) and there is every reason to expect magnetic amplitudes to decrease The observed dramatic decrease in amplitude of the axial anomaly as the spreading axis shoals toward Iceland (Fig 1) means, if our telechemistry argument[1] is valid[4], that the degassing effect overpowers the opposing effect expected from the increasing[3,8] Fe and Ti content Fluctuations in Pleistocene sea level would have smeared out the decline in magnetic amplitudes beyond that expected from sampling only post-glacial basalts Glacial erosion could also have removed some of the magnetised layer

Unfortunately, the Ti and Fe concentrations decrease linearly south-westwards, away from Iceland so steeply that there is only a small section of the Reykjanes Ridge deeper than the degassing depth (as defined by reduced S concentrations) but still anomalously high in Ti and Fe In that small section Ti and Fe are only slightly above the normal levels occurring further south on the Reykjanes Ridge, and any increase in anomaly amplitude in that small section may be within the noise level The observed increase of amplitude as Iceland is approached from either side seems to be accounted for largely by the changing source depth We are investigating this

As degassing should not, in general, affect concentrations of rare earths, trace and minor elements such as Fe and Ti, and as the Reykjanes Ridge is the only geochemically anomalous region for which detailed data exist, we adduced the Reykjanes chemical data[3] in support of our hypothesis, in spite of the absence of high magnetic amplitudes This absence does not demand a new *ad hoc* hypothesis on our part nor is it an additional latitude as construed by Watkins[9] In fact, low pressure degassing is a second kind of 'magnetic telechemistry', which reduces J and therefore magnetic amplitudes It is uncommon because the mid-oceanic spreading axis rarely approaches within 500 m of sea level.

Watkins implies that the differences on magnetic amplitude observed in the Galapagos, Juan de Fuca and Pacific-Antarctic areas[1] are perhaps explained more plausibly by differences in thickness of the magnetised layer, mode of emplacement, oxygen fugacity, degree of quenching, and oxyexsolution The thickness of the magnetised layer is not measured readily as its meaning in terms of seismically determined crustal structure is still somewhat obscure[11] In any case, there is no evidence that the crust in the anomalous areas discussed is thicker than elsewhere We are talking about regional bulges of oceanic crust produced by simple spreading, not about aseismic ridges with thickened crusts If a thicker layer provides the explanation, deep tow magnetic/seismic profiling would reveal the same apparent magnetisation of minor basement features in both areas But profiles collected in the Galapagos area show that the high-amplitude region has more intensely magnetised basement irregularities[11], and Fe/Ti basalt also has higher J values in the few dredge samples available[11] Basement depth is generally continuous across the regional boundary, and both kinds of lavas are exposed to the same physical environment when they erupt There seems no reason to postulate that the other factors cited by Watkins are necessarily important variables but it is also hard to show that they are not The advantage our telechemical argument has is that chemical differences have been reported from all hot spots so far studied

Differences in the mode of emplacement cannot be discounted entirely, especially in the case of the Juan de Fuca Gorda ridges with their different morphologies and different levels of seismicity[12] If the zone of active igneous activity is broader, anomaly amplitudes fall, and the resolution of short-period reversal events deteriorates[13,14] It is doubtful, however, whether this mechanism could explain the uniform amplitude difference between the Jaun de Fuca and Gorda ridges In the Galapagos area, no such deterioration is evident and the width of the 'variable dyke zone' hypothesis seems quite unlikely

So until a more plausible hypothesis is advanced, we stick with magnetic telechemistry This does not mean that the two regions (of high and average amplitude respectively) are likely to differ only in their bulk chemistry Nor does it mean that magnetic amplitudes are necessarily a linear function of Ti and/or Fe For example, if the rate of increase of magnetisation decreases with increasing iron content, then relatively uniform, high amplitude provinces, within which there is substantial geochemical variation, would result Should the analysis of rock samples demand such a complex relationship between chemistry and magnetic anomalies—possibly involving additional 'hidden' variables—it would in no way demolish our hypothesis

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The reaction of monkeys to 'fearsome' pictures

THE phenomena a man or animal most needs to know and understand must often be potentially dangerous or discomforting We report here evidence that monkeys, given the opportunity to look at a picture which excites both interest and fear, choose first to look at it and only later, once their interest has abated, to avoid it

We have measured the preference for a visual stimulus by allowing the monkeys to choose between looking at the test stimulus and a blank white screen of the same subjective brightness In earlier experiments[1,2] we found that the response to an informative but affectively neutral stimulus, for example a repetitive loop of cartoon film, was typically an initial strong positive preference which declined within a few hundred seconds to relative indifference By contrast, the response to an uninformative but unpleasant stimulus, such as a plain field of red light, was a strong and stable aversion When a stimulus was both informative and unpleasant, for example a black and white film loop projected through a red filter, the monkeys' 'interest' overrode—so long as it lasted—their 'unpleasure' The purpose of the present study was to find out what would happen with stimuli which now were deliberately chosen to be both informative and 'scaring'

A television screen was used for displaying the stimuli, which were recorded on video-tape loops so that each action sequence was repeated approximately every 10 s We made up 15 potentially fear-evoking stimuli, selected partly on the basis of our own hunches and partly on the basis of Hebb's suggestion[3] that 'anomalous' objects give rise to fear in primates The stimuli included a toy snake, burning paper, a lavatory brush, a mop-head wearing a human mask and

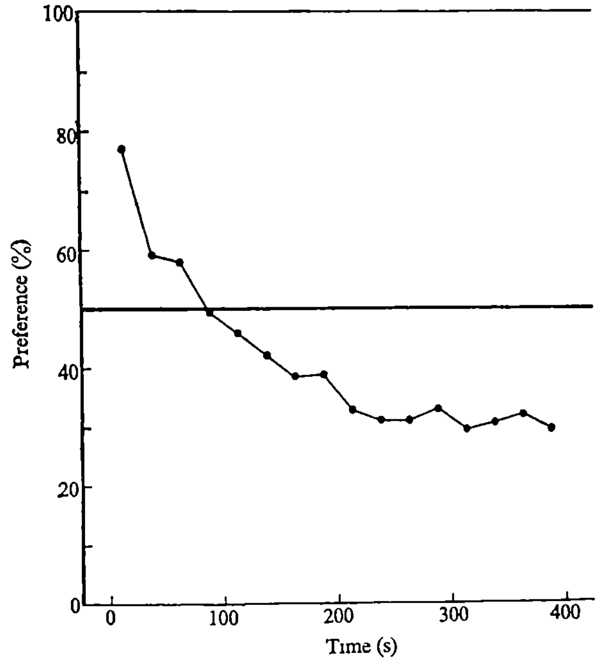

Fig 1 Preference for 'fearsome' pictures (preference measured as the ratio of the total time spent with the test stimulus to the total time spent with either the test stimulus or the blank white screen)

other objects similarly bizarre Each object was given some kind of life-like motion

The method for measuring the monkeys' preferences has been described in detail in earlier papers[1,2] The monkey sat in a small dark chamber with a television screen (37 cm × 30 cm) at one end, taking up most of the wall On the screen could be displayed either the test stimulus or a blank white field of the same subjective brightness The monkey controlled the presentation of the two alternative stimuli by pressing a button successive presses on the button produced the two stimuli in strict alternation, the stimulus staying on as long as the monkey held the button down The test was terminated after 400 s exposure (at which point two peanuts were delivered) The monkeys worked eagerly and generally completed the test in under 500 s, alternating rapidly between the two stimuli (on average about 30 alternations per 100 s of exposure)

The subjects were five young male rhesus monkeys (*Macaca mulatta*) All had taken part in previous experiments on preference, in the course of which they had completed several hundred tests and had become thoroughly familiar with the apparatus and with the fact that strange stimuli were likely to be presented to them They had, however, never before been deliberately exposed to fear-evoking stimuli

The monkeys were tested with two new stimuli each day over a period of three weeks It was immediately apparent from the results that it was not so easy to scare the monkeys as we had imagined In fact in many of the tests there was no sign that the monkeys were anything other than interested in the stimuli Since our aim was specifically to study the interaction of interest and fear, we needed to select for analysis only those tests in which we could be reasonably sure that the monkeys were indeed afraid Independent indices of distress (cries, urination and so on) showed that the stimuli which the monkeys found most upsetting were, as might be expected, those to which they showed the greatest overall aversion We decided therefore to treat any test in which the monkey's preference was predominantly negative as a case of the monkey being scared, and accordingly we defined a stimulus as being 'fearsome' to a particular individual if he chose to look at it for less than 200 s of the 400 s test By this criterion, 13 of the 15 stimuli turned out to be fearsome to one or more of the monkeys, while the five individual monkeys were scared of eight, seven, seven, three and three of the stimuli, respectively

Figure 1 shows the typical pattern of preference for 'fearsome' stimuli, as defined above, in each successive 25 s of the test To obtain this graph we took for each monkey, the mean of his preference for those stimuli which were fearsome to him and then the mean of these means for the five animals The point to notice is the time course The characteristic pattern was for aversion to appear only after a short-lived positive preference This pattern was fully borne out by the data from the individual tests of the 28 tests in which the stimulus proved fearsome, the preference, taken over each successive 50 s, was positive for the first 50 s in 24 instances and negative for the last 200 s in every instance

The pattern of preference was very similar, at least in quality, to that obtained with the red film loops in the earlier study[2] We showed previously that the response to 'red films' could be accurately accounted for by a mathematical model which treats 'interest' and 'pleasure/unpleasure' as two separate factors which interact to determine behavioural preference, subject to a combinatorial rule which gives precedence to 'interest' Our results suggested that the response to fearsome pictures might likewise be dually determined, and the intriguing possibility arose that we might be able to 'synthesise' the curve of Fig 1 by using suitably chosen 'compound' stimuli in which we could separately identify components of interest and unpleasure

To this end we undertook a second experiment in which we presented the monkeys with non-fearsome television pictures to which an unpleasant auditory stimulus ('white noise') was added The non-fearsome pictures consisted of 10 s video loops of relatively bland material, chosen to be roughly comparable in information content to the fearsome

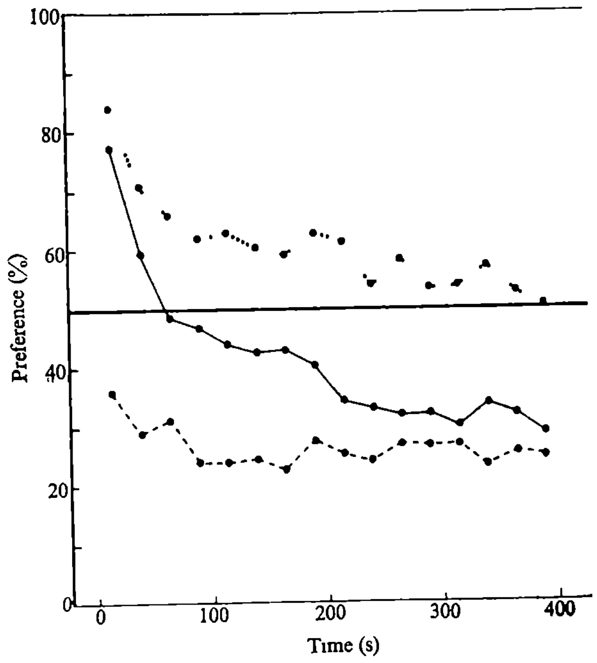

Fig 2 Preference for, ● ···· ●, 'picture only' (a choice between a non-fearsome, silent, video loop and the blank white screen), ● ---- ●, for 'noise only' (a choice between the blank white screen plus white noise and the blank white screen without the noise), ●——●, for 'picture+noise' (a choice between a non-fearsome video loop plus white noise and the blank white screen without the noise) Monkeys were given 10 tests in each category, taken in rotation Graphs show the means of the mean preferences of the five animals

pictures The white noise was set at an intensity level of 68 db (16 db above the background level of the testing chamber) which preliminary observations indicated would create an appropriate degree of unpleasure

The monkeys were given three categories of tests 'picture only', 'noise only' and 'picture plus noise' As Fig 2 shows, the response to the pictures only was an initial strong positive preference which declined towards indifference, that to the noise only was a steady aversion (increasing slightly over the first 100 s of the test), that to the pictures plus noise was an initial positive preference which then turned into a marked aversion In line with the predictions of the mathematical model, the response to pictures plus noise could be almost perfectly fitted by a theoretical curve computed from the separate responses to pictures only and noise only[1]

Comparison of Figs 1 and 2 illustrates the close correspondence between the response to the fearsome pictures and that to the non-fearsome pictures plus noise It seems fair to say that the 'fearsomeness' of the fearsome pictures had on average the same effect as 68 db of white noise But we believe there are grounds here for a stronger assertion, namely that at a causal level fearsomeness influences behavioural preference in the same way as noisiness (or redness) through the evocation of a common factor of 'unpleasure', a factor which is strictly subservient to 'interest'

In functional terms the lesson of these results seems clear the benefits that come from increased understanding outweigh the immediate rewards of a comfortable life

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Behavioural thermoregulation in a wolf spider

LABORATORY studies have shown that wolf spiders (Lycosidae) may prefer temperatures above the ambient air temperature of their normal environment[1,2] They are commonly active on clear days in winter and may repeatedly move in and out of the sun In addition, they carry their egg sacs on their spinnerettes and apparently incubate them in the sun[3,4] While the observations suggest that these spiders may exhibit behavioural thermoregulation, it has not been demonstrated

During a study into the ecological energetics of the large, burrow-inhabiting wolf spider *Geolycosa godeffroyi* (L Koch 1865) in the Australian Capital Territory[5], continuous temperature records were obtained of both the egg sac temperature and the temperature of the cephalothorax of the spiders

Egg sac temperature was measured by implanting a temperature transmitter into the egg sac after the removal of about 70% of the eggs The egg sac was returned to the female in the field and the transmissions monitored during the daylight hours The resulting magnetic tapes were transcribed at intervals and records obtained from six individuals The temperature of the spiders was recorded in the field by implanting a fine thermocouple junction into the cephalothorax and recording the temperature with an electric thermometer with an external reference junction at 0° C The fine thermocouple lead (2 3 mg cm^{-1}) allowed the spider free movement within the burrow and through its normal activity range The ambient air temperature was recorded from the dry bulb of an aspirated hygrometer placed 1 cm above the ground surface Temperatures were recorded for 24 h periods from spiders weighing 0 1–1 5 g from February to November 1972

Figure 1 shows two representative records of the cocoon temperature in summer Figure 1*b* is the record for an intermittently overcast day showing that the egg sac could achieve a considerable temperature excess[6] In Fig 1*a* the spider provided its own control At A the spider was placed with its 'bugged' cocoon next to its burrow which was partly occluded by debris The spider began to clear the debris from the burrow but at B dropped the egg sac in full sunlight outside the burrow and continued with its work The sun was masked by cloud at C and when the spider had cleared the burrow it recovered the egg sac (D) and entered the burrow Between D and E the sky cleared and the spider began to sun the egg sac at E The burrow was lightly shaded by trees after 1500h but was bathed in full sunlight at 1800h, just before sunset The spider maintained a fairly constant egg sac temperature in full sunlight (1200h to 1500h) but the temperature excess potentially reached was considerably greater (A to E in Fig 1*a*) than that maintained by the behaviour of the spider

Figure 2 shows three representative records of the cephalothorax temperature of the spiders at different times of the year Figure 2*b* represents part of the record obtained on a subfemale on March 13, 1972 At 0600h the spider was still benefiting from the thermal lag in the soil temperature profile and by remaining at the bottom of the burrow maintained its temperature 8° C above the ambient air temperature As the ambient temperature rose in the morning the spider came to the top of the burrow and maintained a temperature close to the ambient temperature until the sun struck the burrow entrance at about 1000h The body temperature rose rapidly to about 40° C when

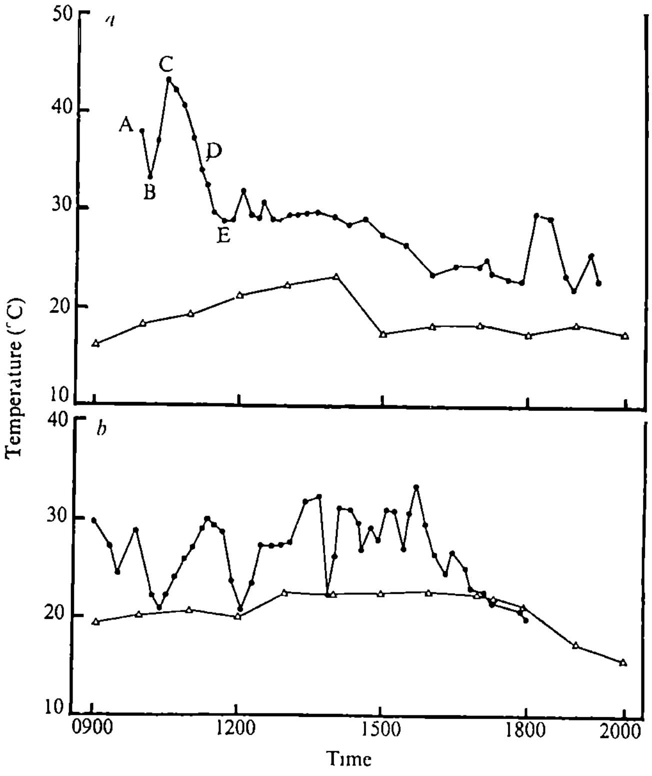

Fig 1 Egg sac temperatures of *G godeffroyi* obtained from implanted temperature transmitters (●) and ambient air temperatures (△) The letters A–E are explained in the text

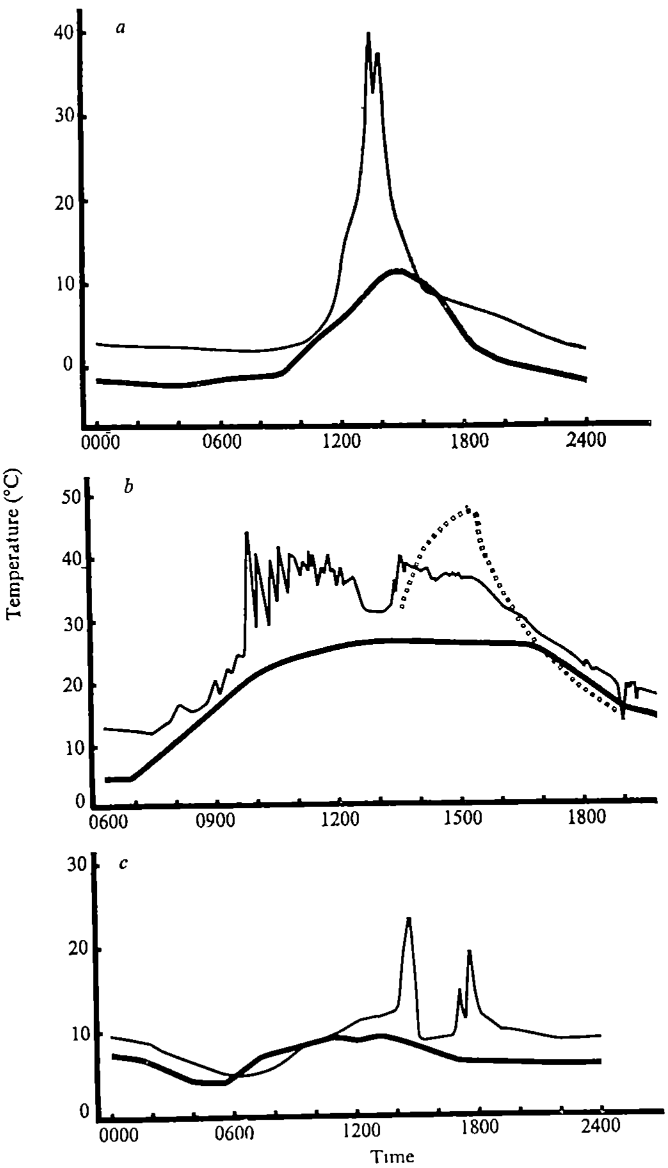

Fig 2 Cephalothorax temperatures of *G godeffroyi* obtained from implanted thermocouples (———) compared with ambient air temperatures (▬▬▬) and dead spider temperatures (☐) The records were obtained in, *a*, midwinter (June 25, 1972), *b*, early autumn (March 13, 1972) and, *c*, late winter (September 13, 1972)

the spider retreated into the shade of the burrow This pocess continued until about 1200h when the sun was partially obscured by cloud until 1330h The ambient temperature dropped after 1600h and the spider retired down the burrow, save for some minor excursions up the burrow at about 1900h, and again maintained its temperature above the ambient air temperature At 1400h a freshly killed spider of similar size was placed on the grass next to the burrow and its temperature monitored The temperature excess obtained by the dead spider greatly exceeded that of the live spider and shows the controlling effect of the spider's movement within the burrow on the body temperature and showing, at night, the moderating influence of the burrow The finer control over the body temperature in the afternoon was characteristic and may result from the spider adopting thigmothermic as well as heliothermic regulation This is supported by the more gradual drop in temperature in the afternoon after the ground had been thoroughly heated compared with the rapid temperature changes in the morning and in winter (Fig 2*a* and 2*c*) whenever the sun was obscured

Figure 2*a* represents the temperature of a spider on a clear winter's day While the ambient air temperature reached 11° C, the spider's temperature rose rapidly to 39 7° C when the sun was on the burrow, resulting in a temperature differential of 30° C at 1345h At night the spider's temperature fell to only 1 8° C in spite of the ambient temperature falling to −2 3° C Figure 2*c* is the record from a spider on a generally overcast day in early spring and shows the immediate use the spider made of radiant energy when the sun struck the burrow at 1300h and 1800h

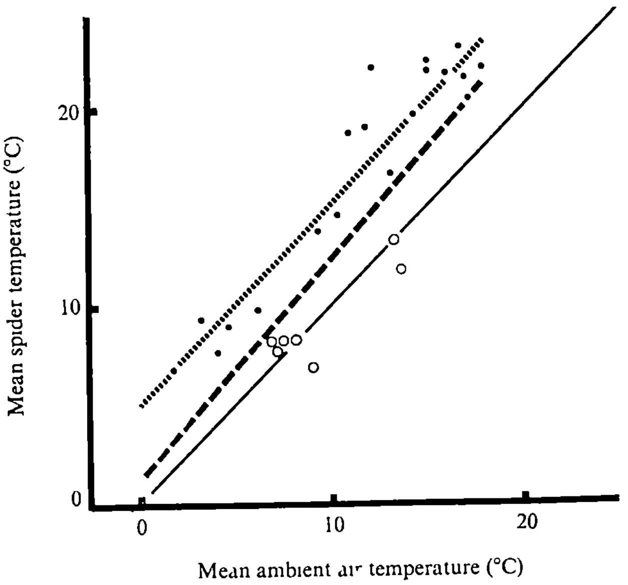

Fig. 3 The relationship between the mean daily cephalothorax temperature and the mean daily air temperature for each of 26 individual *G godeffroyi* recorded throughout 1972 The null line (———) assumes that the ambient and spider temperatures are equal The records are divided into those obtained on clear days (●) and overcast days (○) and the regressions shown for all days (– – – –) and clear days (- - - - -)

In Fig 3 the mean daily spider and ambient temperatures are plotted for the 26 d of recording throughout 1972 The slope of regression through the data for the clear days only is not different from a slope of 1 0 (t_{19}=0 845, 0 5>P>0 4) On clear days throughout the year the spiders maintained their body temperature an average of 4 6° C above the ambient air temperature by basking in the sun during the day and retreating down their burrows at night

The similarity between the thermoregulation in *G godeffroyi* and that found in some lizards[6–8] is striking, the main difference lying in the finesse of the control in the plateau temperature While the lizards exhibited fine control at the upper temperature, that of the spiders was erratic, probably due to their low thermal capacity

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Functional reinnervation in kitten visual cortex

MOST cells in the adult cat visual cortex, and even in the very young kitten can be activated by visual stimuli presented to either eye[1-3] The retention and refinement of these normal binocular connections depends critically on simultaneous correlated visual experience through both eyes early in life If a kitten is deprived of vision in one eye during a 'sensitive period', which stretches from about 3 weeks to 3 months, the normal pattern of functional connections is grossly disrupted, the deprived eye loses its influence and most cells can only be driven by stimuli through the experienced eye[4] Blakemore and Van Sluyters[5] have recently shown that this situation can be partly or totally reversed by covering the previously experienced eye and opening the deprived eye, and allowing that eye a substantial period of visual experience Thus one set of afferent terminals, robbed of almost all influence in the visual cortex, can re-establish working connections We have now analysed the form and rate of this functional reinnervation

In six kittens the lids of the right eye were sutured together at or before the time of natural eye opening (normally 6–10 d) Reverse suturing was performed at the age of 5 weeks the lids of the right eye were opened and those of the left eye sutured shut All precautions were taken to ensure that the animals never received simultaneous binocular vision As a control, one kitten was monocularly deprived in the right eye for 5 weeks, and recordings taken immediately

Animals were prepared for electrophysiology under barbiturate (Brietal) or steroid (Althesin) anaesthesia They were then paralysed with Flaxedil and artificially respirated with 75–80% nitrous oxide[6] The corneas were covered with contact lenses and 3 mm artificial pupils, and refractive errors corrected with supplementary lenses Single units in the visual cortex were isolated with tungsten-in-glass microelectrodes[7], and their action potentials were conventionally amplified and displayed

All penetrations were later shown histologically to be largely or wholly within area 17 The electrode was driven down the medial bank of the postlateral gyrus, obliquely crossing a large region of cortex The first cells encountered in each penetration had receptive fields within 5° of the area centralis and all receptive fields were within 15°

We studied the responses of neurones to flashing and moving patterns back projected on to a tangent screen 57 cm from the cat's eyes Receptive fields were classified into one of five groups orientation selective[1] (simple, complex or hypercomplex), pure direction selective, orientational bias, nonoriented or visually unresponsive[5] Units with the brisk response, fibre waveform and clear concentric receptive field organisation of afferent fibres from the lateral geniculate nucleus were excluded from the analysis For all cells that were excited by visual stimuli we assessed the relative responsiveness through the two eyes and classified them using Hubel and Wiesel's seven-point scale of ocular dominance[1] Cells in groups 1 and 7 are solely monocularly driven, group 1 by the eye contralateral to the recording site, group 7 by the ipsilateral eye Group 4 cells are equally influenced by the two eyes Groups 3 and 5 are slightly more strongly driven by the contralateral and ipsilateral eyes respectively, groups 2 and 6 are strongly dominated by the contralateral and ipsilateral eyes respectively All recordings were made from the right hemisphere, contralateral to the initially experienced left eye

The ocular dominance histograms in Fig 1*a* show the results for 27 cells from the control animal, monocularly deprived in the ipsilateral eye until recording at 5 weeks of age, and for 189 cells from six kittens reverse sutured at 5 weeks and allowed various periods of time in which to use the previously deprived ipsilateral eye As expected, the first histogram shows that 5 weeks of monocular deprivation silenced virtually all connections from the deprived ipsilateral eye almost all cells are in group 1 But 3 d after reverse suturing many neurones could be driven by the initially deprived eye After only 1 week the process was about half complete, and within 3 weeks there was almost no influence from the previously dominant eye These data are presented graphically in Fig 1*b* where the proportion of cells dominated by the initially deprived eye is plotted against the number of days after reverse suturing

We observed striking changes in the functional architecture of the cortex during reversal of monocular deprivation Cells were clustered into distinct regions dominated by one eye or the other—the 'ocular dominance columns' of Hubel and Wiesel[8] We judged the dimensions of these columns by the distances between obvious switches in ocular dominance during each long penetration The relative size of the columns seemed to change from animal to animal in an ordered manner In kittens where the two eyes commanded roughly equal numbers of cortical cells, the columns for the two eyes were of about the same size, in the more extreme cases the regions devoted to the eye that, overall, dominated fewer cells were smaller and sparser

Thus, after reverse suturing, areas dominated by the initially deprived eye appear, then expand and ultimately occupy the whole cortex Such an orderly anatomical progression suggests that this physiological recapture may represent actual growth and reorganisation of afferent

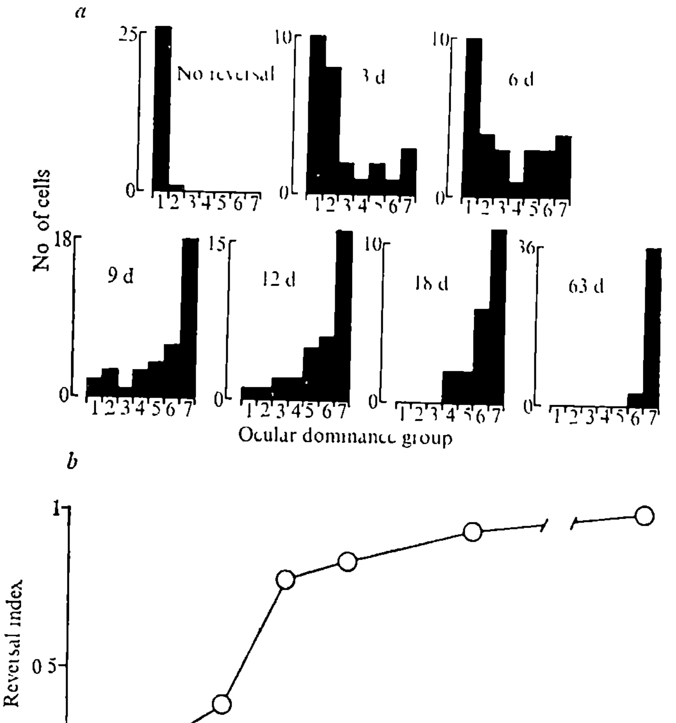

Fig 1 *a*, The ocular dominance histograms of 216 visually responsive cells from the series of seven kittens recorded at varying times after reversal of lid suture The ocular dominance classification is described in the text *b*, The process of reversal is shown graphically, plotting reversal index against days after reverse suturing The reversal index is calculated by dividing the number of cells dominated by the previously deprived eye (groups 5, 6 and 7) by the total number of cells terminals, not merely the strengthening of existing but silent synapses. If so, it implies that the mammalian central nervous system, at least early in life, has some powers of rapid regeneration.

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Intercellular gap junctions in pigment epithelium cells during retinal specification in *Xenopus laevis*

DURING embryonic development the cells of the retina acquire positional information which predisposes the future optic axons to connect with specific loci in the optic tectum. In *Xenopus* embryos the eye becomes irreversibly polarised along the naso-temporal and dorso-ventral axes by stage 32[1], after which it is insensitive to any specifying influence previously exercised by the tissues surrounding the eye[2].

In a recent electron microscope study[3] of the developing *Xenopus* retina we described the fine structure of the developing retina between stages 26 and 36. We noted then, the presence of intercellular gap junctions which occurred throughout the retina up to stage 30/31. Beyond stage 32 these junctions could only be found at the periphery of the retina where stem cells contribute new cells to the growing retina[4]. Hence the disappearance of these gap junctions from the central portion of the developing retina appears to be correlated with the time of retinal specification.

We have recently re-examined our data on the developing *Xenopus* eye and noted a significant feature which we failed to report in our original paper. Namely, that cells of the embryonic pigment epithelium, as well as cells of the neural retina, establish gap junctions with one another throughout the eye prior to stage 30/31. We also observed gap junctions between retina and pigment epithelium (Fig. 1). After stage 32 these gap junctions disappear from the centre of the eye but persist for some time in the periphery.

Many results support the view that the flow of molecules through cell junctions may be important in the control of cellular growth and differentiation[5]. In our previous paper we suggested that the disappearance of gap junctions from the central part of the eye and the time of retinal determination might be causally related. Our present observations on the distribution of gap junctions between developing retina and pigment epithelium prior to stage 32 suggests that not only the retina, but also the pigment epithelium, may be subject to the specifying influence of the surround through intercellular channels. This is particularly interesting in view of recent indications that pigment epithelium cells from the centre of the eye of an adult newt may pass on positional information to a new regenerating retina[6].

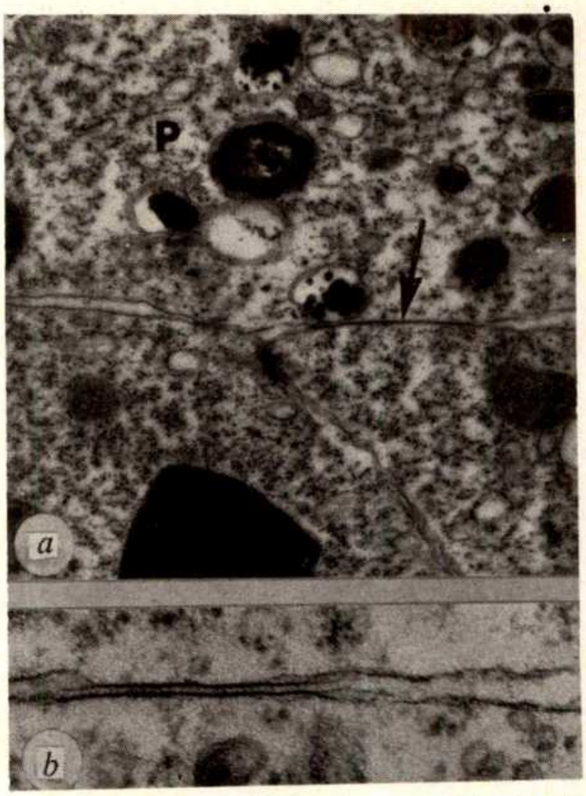

Fig. 1 *a*, The arrow indicates an intercellular junction between a germinal pigment epithelial cell (P) and a retinal cell at stage 26. (×24,000); *b*, A similar junction at higher power illustrating the characteristic narrow gap of 3–9 nm. (×94,000).

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Specificity of regenerating optic fibres for left and right optic tecta in goldfish

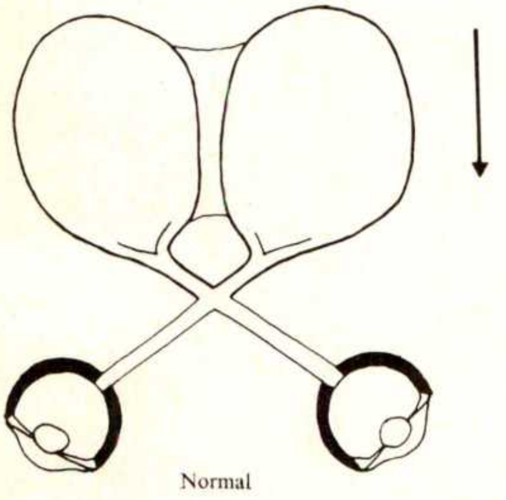
RECENT studies[1] on the regeneration and development of the visual nervous system of inframammalian vertebrates have concentrated on the topographic patterning of retinotectal connections, neglecting the problem of what specifies the pathways of large tracts. Embryologically the eye is an outgrowth from the ipsilateral side of the brain, but during development the optic nerve fibres cross the midline and terminate on the opposite side where they "mirror" the topographical disposition of their retinal ganglion cells. Sperry[2,3] proposed that the precise patterning of such neuronal circuits resulted from specific affinities between individual nerve fibres and their target loci within the brain, but recent evidence[4] suggests that what is important is the preservation of the overall pattern of fibre connections, rather than the position occupied by individual terminals[5].

Investigation of the pattern of recovery following reinnervation of muscles in fish[6,7] suggests that competitive interaction between different nerve fibres may in some species lead to the selection of correct connections. We now report some preliminary results where both optic nerves in goldfish were directed into the same tectum to test for competitive interaction for occupancy of the same tectal site.

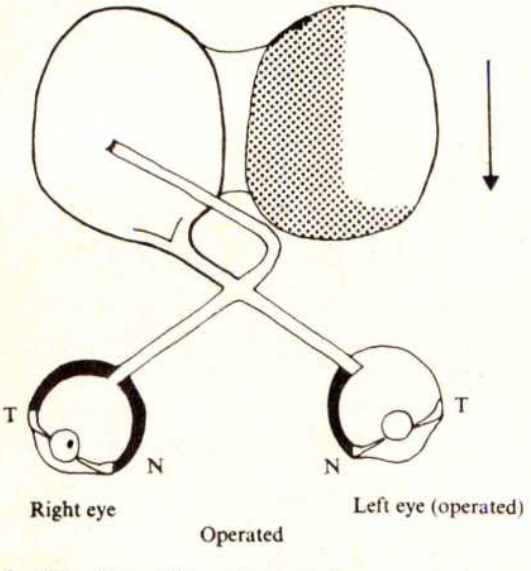
Goldfish were anaesthetised in a 0.4% solution of MS222 (Sandoz), and mounted in a holder which allowed the gills to be continuously perfused with water, or with a solution of MS222, as required. Two operations (Fig. 1) were then performed on the fish. The tectum of the anaesthetised fish was exposed by opening a cranial bone flap which was replaced after surgery. A small tungsten wire hook was then inserted under the left optic tract, optic fibres stripped away from the left tectum, and the length of nerve brought right over to lie across the right optic tectum. A medial strip from the left tectum was excised by aspiration along its whole length to prevent the nerve from growing back to the original tectum and the bone flap replaced. A second operation was then performed to remove the temporal hemiretina from the fish's left eye (other experiments where a hemiretina was removed from each eye will be reported later). In each case an incision was made to remove the hemiretina by cutting along the vertical meridian at the back of the eye. Bleeding was reduced by a noose of silk thread slipped around the optic nerve. After the operation the fish were returned to individual home tanks where they were kept for a year before recording. The length of the postoperative period was to allow any possible neuronal respecification[8] to occur before investigation.

For electrophysiological recording anaesthetised fish were mounted in their holder, and the tectum exposed. One eye was centred on a projection perimeter (the other eye was always covered with a plasticine occluder) and the visuotectal representation for that eye on the right tectum mapped with a Woods-metal microelectrode[9]. The same procedure was then repeated for the other eye.

In 21 of the 24 fish which survived the recovery period, the nerve from the right eye failed to form connections with the ipsilateral tectum and showed a marked tendency to curve back to try and reach the remaining fragment of lateral tectum on the contralateral (and correct) side. Unfortunately the left tectal fragment was often placed too far laterally to enable us to record from it effectively. In three fish, where the operation was successful, one was a control, where in addition to uncrossing the chiasma we removed the nasal hemiretina from the left eye and the temporal hermiretina from the right eye. In this control fish, the representation of the hemiretinas from the right and left eyes, were each confined to their 'correct' half of the right tectum. The other two fish gave results that were similar to one another. Figure 2 shows the tectum of one of them, where we succeeded in getting the optic nerve from the right eye (or at least part of it) to deploy itself in the ipsilateral optic tectum. There was also some suggestion that optic fibres from the right temporal hemiretina had crossed back to the correct (left) tectum via the tecto-mesencephalic commissure. Indeed, a band of what appeared to be newly myelinated fibres was clearly visible at the caudal end of this commissure (Fig. 2). Furthermore, on the right optic tectum, we found no evidence, electrophysiologically, of any representation of the temporal retina from the right eye (Fig. 3). Two zones innervated exclusively by one eye or the other are separated by a zone in which the two projections overlap. The area of overlap seemed to vary in different fish, and it is possible that during the early stages of recovery the two projections may have overlapped completely and only later become segregated from one another.



Normal



Operated

Fig. 1 Operations. The optic nerve from the right eye was uncrossed surgically and diverted to the right tectum, and the temporal half of the retina from the left eye was removed. Shaded region shows area of tectum removed.

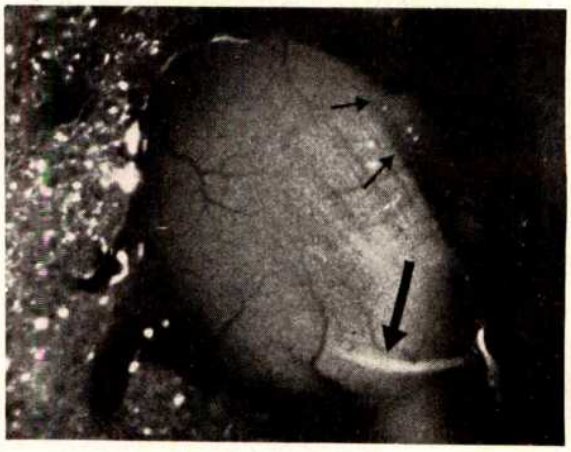


Fig. 2 Large arrow: insertion of the uncrossed optic nerve from the right eye into the ipsilateral optic tectum. A band of newly myelinated fibres can just be seen in the caudal region of the tecto-mesencephalic commissure (small arrows).

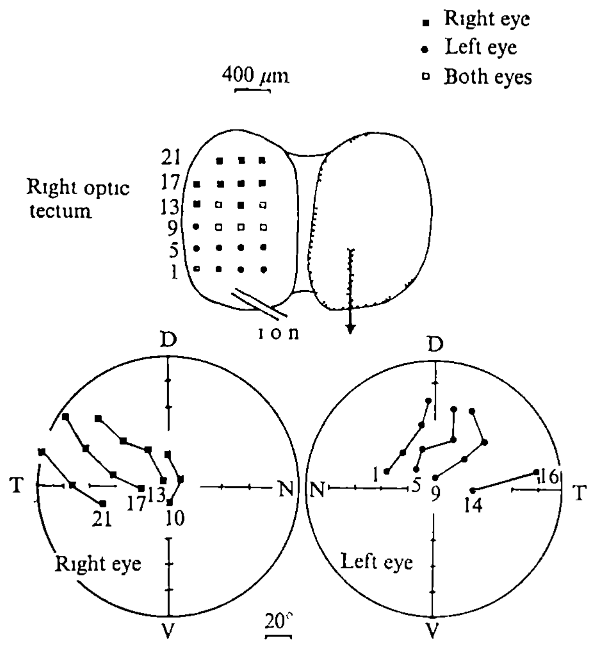


Fig 3 Visual field projections from the right and left eyes onto the right optic tectum Shaded region shows area of tectum removed i o n , optic tract from ipsilateral eye

Previous studies[10] have shown that if a sufficient period is allowed for recovery, the fibres from a nasal hemiretina can spread and occupy a whole normal tectum By contrast, in Fig 3 the projection from the left nasal hemiretina only extends over approximately half the dorsal surface of the tectum Moreover, in this instance the nerve from the contralateral eye occupied the wrong half of the tectum (in the normal contralateral projection[11] optic fibres from the nasal hemiretina occupy the caudal half of the tectum) This suggests that the contralateral nerve fibres may have been displaced forwards by competition for tectal sites with fibres arriving fom the ipsilateral nasal hemiretina

While it is clearly too early to draw any hard and fast conclusions, our observations indicate (1) that regenerating optic fibres may be able to distinguish between right and left tecta, and (2) if both optic nerves are forced into the same tectal field, each may have a tendency to exclude the other from the tectal zone it innervates

There is one further point worth noting Yoon[8] found that in certain conditions, goldfish optic fibre terminals would arrange themselves topographically in an anomalous region of the optic tectum Yoon attempted to reconcile his results with Sperry's original chemoaffinity hypothesis[3] by suggesting that respecification of tectal elements might have occurred after surgery Our study, however, makes this interpretation unlikely While only the nasal half of each retina was represented on the tectum, optic fibres from corresponding retinal loci deployed themselves topographically at different locations of the same unoperated tectum It is difficult to envisage how respecification of tectal elements could account for the development of a duplicate set of 'tectal markers' in an unoperated optic tectum

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Evidence for two mechanisms of sclerotisation in insect cuticle

In many insects the cuticle changes drastically in properties soon after a moult When the insect emerges from the old cuticle the new one is soft, pale, and a major part of the proteins can easily be extracted Gradually the proteins become insoluble, the cuticle becomes harder, and in many cases it also becomes darker The colour may range from a very light amber to dark brown or even black It has been discussed repeatedly whether hardening and darkening are two independent processes or whether they are two results of the same chemical reaction, but no definitive conclusions have been reached[1–6]

During a study of cuticle sclerotisation I have obtained evidence which confirms an earlier suggestion[7] that two mechanisms for forming cross links exist in insect cuticle, one giving a colourless cuticle and the other a dark brown cuticle Both mechanisms can be active simultaneously in the same piece of cuticle and they can utilise the same substrate, N-acetyldopamine In the reaction resulting in a colourless cuticle the β position of the substrate is activated and becomes involved in cross linking of the proteins, while the aromatic ring is involved in the reaction giving rise to a brown cuticle

Both activities were assayed by means of tritiated N-acetyldopamine, for the former assay tritium was located on the β carbon atom of the sidechain and for the latter assay it was restricted to the aromatic ring of the substrate The two substrates were synthesised by treatment of 3,4-dihydroxyphenylethylamine (2^{3}H^{-} ethyl) and 3,4-dihydroxyphenylethylamine (^{3}H^{-} ring), respectively, with acetic anhydride in 10% potassium tetraborate[8] The purified products were diluted with unlabelled N-acetyldopamine to give 10 mM solutions which contained 2 μCi ml^{-1}

Samples of cuticle were obtained from the desert locust, *Schistocerca gregaria*, the silk moth, *Hyalophora cecropia*, and from meal worms, *Tenebrio molitor* All cuticles were carefully cleaned and washed in 1% potassium tetraborate and in distilled water They were divided into the appropriate parts which were incubated in 2 6 ml 0 2 M Na-acetate buffer, *p*H 5 5, 100 μl of either one or the other substrate was added, and the mixture was incubated for 18 h at room temperature The tritium which was split off during this period was determined after passing the supernatant through a small column (0 5 × 1 cm) of activated charcoal, which retains the aromatic substrate but not tritiated water[8] The first millilitre of the eluate was discarded, and from the rest of the eluate 1 ml was taken to be counted The non-enzymatic release of tritium from the substrates was determined in parallel experiments without any cuticle and was used to correct the experimental results The dry weight of the cuticular samples was determined after the incubations by drying them to constant weight at 100° C

Table 1 shows that all the cuticular samples will release tritium from both substrates and that the relative activities vary considerably The various types of locust cuticle which remain lightly coloured during sclerotisation show a much higher activity towards the sidechain of N-acetyldopamine than towards the ring, whereas the mandibles, which are a

Table 1 Amounts of tritium released from ring-^{3}H-labelled and β-^{3}H-labelled N-acetyldopamine (NADA) on incubation with various cuticular samples

Sample	Colour of fully hardened cuticle	n*	Radioactivity (c p m mg^{-1}±s d) released from ring-^{3}H-NADA	Radioactivity (c p m mg^{-1}±s d) released from β-^{3}H-NADA	Ratio ring-^{3}H/β-^{3}H
Schistocerca gregaria					
Adults					
Femur	Colourless with black areas	4	438±136	7,590±1398	0 06
Tibia	Colourless with dark brown spines	4	1,121±190	7,733±1237	0 15
Lateral meso- and metathorax	Colourless with dark ribs	4	571±73	10,052±1250	0 06
Dorsal meso- and metathorax	Light brown	2	1,090	13,126	0 08
Ventral meso- and metathorax	Colourless	2	491	6,106	0 08
Pronotum	Colourless	2	671	10,098	0 07
Dorsal abdomen	Colourless	4	233±105	11,400± 254	0 02
Ventral abdomen	Colourless	2	107	7,789	0 01
Head capsule	Colourless	4	429±112	9,990±1110	0 04
Mandibles	Dark brown	2	1,443	3,000	0 48
Fifth instar larvae					
Femur	Colourless with black areas	5	2,359±123	33,052±620	0 07
Mandibles	Dark brown	3	4,411	4,474	0 99
Tenebrio molitor					
Adults					
Ventral abdomen, young	Dark brown	6	6,064±714	4,005±780	1 51
Ventral abdomen, old	Dark brown	2	272	2,488	0 11
Hyalophora cecropia					
Larval abdomen	Colourless	6	1,888±109	6,504±104	0 29
Young pupae	Dark brown	4	13,177±1808	14,404±1390	0 91
Old pupae	Dark brown	4	10,225±1375	18,881±1331	0 54

The adult locusts were killed 1 d after the moult, their cuticle was thus well sclerotised, while the fifth instar larvae were caught while moulting The young *Tenebrio* adults were caught less than 12 h after the moult and were light brown in colour, whereas the old adults were about 2 week old and were very darkly coloured The young *Hyalophora* pupae had an uncoloured frontal part and a light brown abdomen, and the old pupae were dark brown all over

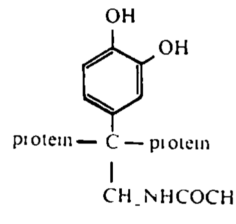
* Number of determinations

dark brown type of cuticle have a relatively higher activity towards the ring part of the substrate No significant differences are observed between those samples of locust cuticle which become melanised and those which do not

The pupal cuticle of *Hyalophora*, which becomes dark brown during hardening, contains high levels of both activities, whereas the enzyme active towards the sidechain of the substrate dominates in the colourless larval cuticle In young *Tenebrio*, where only little sclerotisation has occurred, the enzyme active towards the aromatic ring dominates slightly, but this activity disappears as the cuticle becomes fully sclerotised, and the sidechain directed activity remains

The results indicate that cuticles which become dark brown during sclerotisation contain relatively large amounts of an enzyme which is active towards the ring moiety of N-acetyldopamine, whereas cuticles which remain pale or colourless prefer the sidechain of the substrate

We have in some earlier publications reported on the sclerotisation of locust cuticle[7-9], and our results suggested that the cross links in hard, uncoloured cuticle are



It can be assumed that the enzyme which can remove tritium from the β position of N-acetyldopamine is also responsible for the formation of the cross links[8] The enzyme which removes tritium from the aromatic ring can be assumed to be an *o*-diphenoloxidase oxidising the substrate to an *o*-quinone which will combine with free amino groups with the simultaneous loss of tritium from the ring This enzyme would thus be responsible for the classical type of quinone tanning of insect cuticle first suggested by Prior[1]

The possibility that both enzyme activities reside in a single enzyme cannot be completely disregarded, although it seems difficult to explain how the ratio between the two activities can vary so much between the various types of cuticle if it is not two different enzymes The decrease of only one of the activities as sclerotisation progresses in *Tenebrio* cuticle also indicates that it is two enzymes It will, however, be necessary to obtain both activities in soluble form before this problem can be solved

The results reported here indicate that insect cuticle contains two enzyme activities, both involved in sclerotisation and both utilising the same substrate, but one of them resulting in a hard and colourless cuticle and the other giving rise to a hard and dark cuticle The intensity of the colour will presumably be determined by the relative amounts of the two enzymes in the cuticle and by the possible deposition of melanins, for which a third enzyme can be assumed to be responsible

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Hepatic necrosis caused by furosemide

Furosemide (frusemide) is a diuretic drug frequently used in the treatment of many cardiovascular and renal diseases Its use is contraindicated in pregnancy because of its recognised teratogenic potential[1] The drug also has been reported to potentiate renal injury when used in combination with cephaloridine[2,3]. Here we report that this widely prescribed therapeutic agent is converted by microsomal enzymes in the liver of mice and humans to a reactive arylating metabolite that produces massive hepatic necrosis in mice.

Male, Swiss albino mice (20–25 g, National Institutes of Health stock) were given furosemide (400 mg kg^{-1}, intraperitoneally, in 0.9% saline solution) and killed at various intervals. Paraffin sections of the liver were prepared and stained with haematoxylin and eosin.

Within 3 h of furosemide administration, nucleolar changes were present in midzonal hepatocytes, and single cell necrosis with pyknotic hepatocytes showing eosinophilic degeneration was also occasionally present. By 6 h, necrosis of many hepatocytes was found in the midzonal and centrilobular areas, demonstrated by nuclear pyknosis, karyorrhexis and cytoplasmic eosinophilia (Fig. 1*a*). Early polymorphonuclear cell infiltration was often present in the less severely injured livers. By 12 h, severely damaged livers showed massive necrosis in both the midzonal and the centrilobular areas such that confluent zones of pyknotic, eosinophilic hepatocytes bridged adjacent lobules (Fig. 1*b*). The Küpffer cells, however, were generally normal. At later time intervals, the pattern of necrosis was usually centrilobular although occasional sections had entirely midzonal necrosis with a collar of viable parenchymal cells remaining around the centrilobular and periportal veins. Necrosis was greatest at 24–48 h; regeneration was evident by 48–72 h.

The histological slides were coded and the severity of the liver necrosis produced by furosemide was determined by two observers blind to the code, as previously described for other hepatotoxins[4]. Furosemide-induced hepatic necrosis was dose dependent (Table 1). After a single furosemide dose of 200 mg kg^{-1}, only slight necrosis (+) was observed in 16% of animals. After administration of 300 mg kg^{-1}, necrosis was present in 68% of mice and was more extensive (++ and +++). After 400 mg kg^{-1}, 92% of mice had necrotic livers and the severity was (++ to ++++) in 60% of the animals.

Table 1 Extent of hepatic necrosis in mice 24 h after various intraperitoneal doses of furosemide

Dose of furosemide (mg kg^{-1})	No. of animals	% Mortality	Extent of necrosis* O	+	++	+++	++++
			%	%	%	%	%
100	25	0	100	0	0	0	0
200	25	0	84	16	0	0	0
300	50	2	32	48	16	4	0
400	50	4	8	32	36	18	6
500	25	20	0	5	40	35	20

* Extent of necrosis (determined as previously described[4]): O, absent; +, necrosis of less than 6% of parenchymal hepatocytes; ++, necrosis of 6–25% of hepatocytes; +++, 26–50% of hepatocytes; ++++, greater than 50% of hepatocytes.

Alterations in hepatic blood flow with resulting hypoxia may play a role in the pathogenesis of centrilobular liver damage. Therefore, we considered the possibility that the hepatotoxicity produced by furosemide might have been caused by hypoxia resulting from massive diuresis with subsequent decrease in the vascular volume. Furosemide-treated mice were never in shock, however, nor showed respiratory depression during our studies, and other diuretics, such as chlorothiazide and ethacrynic acid, did not cause hepatic necrosis even after LD$_{50}$ doses. Moreover, the dose of furosemide required to produce necrosis was much greater than that which usually produces maximal diuresis[3] and post-treatment with Ringer's lactate in 5% glucose (12.5 ml kg^{-1}, intraperitoneally, at 1, 2, 3 and 4 h after administration of furosemide) did not prevent the lesion. In addition, the initial hepatic lesion occurred midzonally, not centrilobularly, and was present a few hours after drug administration. Finally, the liver damage is probably caused by a metabolite of furosemide rather than the parent drug, because necrosis was prevented when the metabolism of furosemide was inhibited by pretreatment of mice with three different types of cytochrome P–450 enzyme inhibitors: piperonyl butoxide, cobaltous chloride and α-naphthylisothiocyanate.

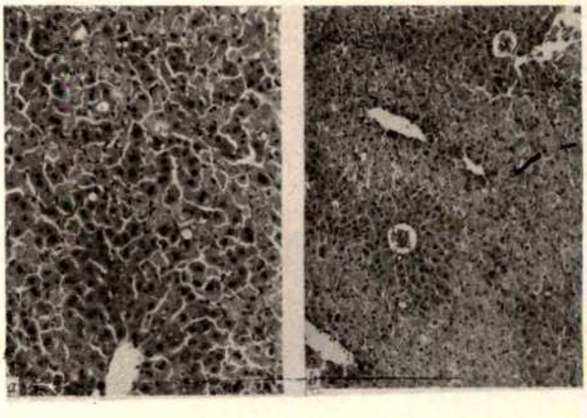

Fig. 1 Furosemide-induced liver necrosis in mice after an intraperitoneal injection of 400 mg kg^{-1} of furosemide. Sections of liver stained with haematoxylin and eosin. *a*, Midzonal hepatic nercosis 6 h after furosemide (×98); *b*, centrilobular-midzonal hepatic necrosis 12 h after furosemide (×44).

In a further series of experiments we studied the enzyme-dependent covalent binding of furosemide to hepatic macromolecules both *in vitro*[5] and *in vivo*[6]; this technique can be used as an index of the formation of an arylating metabolite of a drug[7,8]. Covalent binding *in vitro* of ^{3}H-furosemide (generally labelled) or ^{35}S-furosemide to hepatic microsomal protein required oxygen and a NADPH generating system and was inhibited by an antibody against NADPH-cytochrome *c* reductase and by a 9:1 carbon monoxide : oxygen atmosphere. Thus, the formation of an arylating metabolite of furosemide was mediated by a cytochrome P–450-dependent mixed-function oxidase in liver. We also found a furosemide metabolite bound to hepatic protein *in vivo* to about the same extent (1.5 nmol mg^{-1} protein) as we had previously found for the reactive, hepatotoxic metabolite of acetaminophen (paracetamol)[7].

Evidence of the covalent nature of the furosemide binding was obtained by hydrolysis with pronase of the solvent-extracted protein precipitate and isolation of the radiolabelled compound bound to single amino acids and peptide fragments. Pretreatment of mice with piperonyl butoxide, cobaltous chloride or α-naphthylisothiocyanate almost completely abolished both the *in vivo* covalent binding of furosemide and furosemide-induced hepatic necrosis. The covalent binding occurred a few hours before the onset of histologically recognisable necrosis and before biochemical changes in the hepatocytes (such as, decreased cytochrome P–450-dependent drug metabolism and decreased protein synthesis[8]). These findings lead us to conclude that the arylation of liver macromolecules by a reactive furosemide metabolite is causally related to the development of furosemide-induced hepatic necrosis.

Another important finding was the presence of a dose threshold for the covalent binding and liver necrosis produced by furosemide[8]. Only small amounts of covalent binding and no necrosis occurred until a dose of 100 mg kg^{-1} was exceeded. Studies on the metabolism, distribution and reversible plasma protein binding of furosemide after non-toxic and toxic doses suggest that this threshold may result from a saturation of the organic anion binding sites on plasma proteins after toxic doses of furosemide[8]. More free furosemide would then be available to the liver for metabolism. Unlike the dose threshold for acetaminophen-induced liver necrosis[10], the furosemide threshold is not the result of a protective role of glutathione since furosemide did not deplete hepatic glutathione.

The hepatic injury produced by furosemide (N-(2′-furylmethyl)-4-chloro-5-sulphamoylanthranilic acid) apparently results from the metabolic activation of the furan ring, possibly by an epoxidation similar to that proposed for the hepatocarcinogenic dihydrofuran aflatoxins[11]. For example, furosemide radiolabelled with tritium in its furan moiety was bound covalently to hepatic microsomes in the presence of oxygen and NADPH to the same extent as furosemide radiolabelled specifically with ^{35}S in its sulphonamide moiety, demonstrating that the bound metabolite contained both parts of the furosemide molecule. To determine where the binding occurred on the molecule, the metabolite-protein conjugates isolated from the liver were hydrolysed in mild acid conditions (*p*H 4.5) that split furosemide into its methylfuran and sulphamoylanthranilic acid portions. The binding of furan-radiolabelled furosemide to protein was unchanged whereas the binding of ^{35}S-labelled furosemide was lost. Thus, the metabolic activation of furosemide must have occurred on the furan ring.

Finally, we have compared the hepatotoxicity of structural analogues of furosemide and have shown that the hepatic lesion can be reproduced by such simple furans as 2-hydroxymethylfuran, 2-acetylfuran and even furan itself (Table 2). Moreover, pretreatment with phenobarbital shifts the zone of necrosis produced by furosemide and by other furan-containing compounds such as ngaione and 3-hydroxymethyl furan (N,N-diethyl)-carbamate[12,13] from a centrilobular to midzonal distribution in mice. Furosemide, furan, 2,3-benzofuran and certain other furano compounds cause toxic lesions in the kidney (Table 2) in addition to the liver, whereas ipomeanol and other furan analogues selectively produce lung damage and pulmonary oedema[14,15]. Thus, a variety of tissue lesions seen after furan-containing compounds may result from a metabolic activation similar to that proposed for furosemide. Extension of these studies to thiophene, an analogue of furan in which the oxygen atom in the five-membered ring is replaced with sulphur, has shown that several thiophene-containing compounds also produce massive hepatic or renal necroses in animals (J.R.M., W.Z.P., J.A.H. and D.J.J. unpublished). Studies are underway to determine whether the nephrotoxicity produced in humans by cephaloridine and cephalothin results from the metabolic activation of the thiophene nucleus in these antibiotic drugs.

The implications of the present findings for the clinical use of furosemide are uncertain. Extensive clinical experience with furosemide has not resulted in reports of liver damage; presumably therefore, the drug is not hepatotoxic at the usual therapeutic doses (40–600 mg d^{-1}). The enzyme pathway responsible for the formation of the toxic metabolite, however, is apparently present in humans because human cadaver microsomes, similar to mouse microsomes, can convert furosemide to an arylating metabolite (J.R.M., W.Z.P., J.A.H., and D.J.J., unpublished). The possibility arises, therefore, that the remarkable safety of furosemide after low therapeutic doses may result from the presence in humans of a dose-threshold phenomenon similar to that found in mice. If this is so, then tissue concentrations of furosemide in patients with renal failure probably are much greater in relation to the furosemide dose, since furosemide is eliminated from the body primarily by renal excretion of the unchanged drug[16]. In addition, patients with chronic renal failure often have decreased concentrations of plasma proteins, making more free (nonbound) furosemide available to the liver for metabolism. Since huge doses of furosemide (for example 2,000 mg by rapid intravenous injection) are currently used to treat patients with acute and chronic renal failure[17], the potential hepatotoxicity of this therapeutic regimen should be carefully evaluated. The possible consequences of the formation in humans of an arylating metabolite from furosemide should also be considered, because most compounds that arylate tissue macromolecules *in vivo* can produce neoplasia[18].

Table 2 Liver, kidney and lung toxicity in mice and rats after furan analogues, given intraperitoneally.

Liver*	Dose† (mmol kg^{-1})	Kidney*	Dose† (mmol kg^{-1})	Lung*
Furan	3.5	Furan	3.5	Ipomeanol§
Furosemide	1.1	2-ethyl furan	2.4	
2-Furamide	0.7	2, 3-benzofuran	1.3	
2-Acetyl furan	2.0	2-furoic acid	1.9	
2-Furfurol	3.9			
2-Ethyl furoate	3.6			
2-Methoxy furan	8.4			
Dibenzofuran	5.8			
Ngaione‡				

* Primary site of lesion. However, the other organs frequently manifested damage but to a lesser degree. Moreover, the site of the lesion often was shifted from one organ to another by pretreatments that altered drug metabolism, for example, administration of phenobarbital or piperonyl butoxide (unpublished results).

† Dose producing necrosis of 6–25% of parenchymal hepatocytes or 6–25% of cells of renal convoluted tubules.

‡ Data from ref. 13.

§ Data from ref. 14.

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Red cell 2,3-diphosphoglycerate concentration in man decreases with age

So far there has been no report in the literature suggesting that the chemical composition of the red blood cell changes during the life of an adult healthy human. We have now obtained evidence that in a normal population the red cell concentration of 2,3-diphosphoglycerate (2,3-DPG) decreases with advancing age. 2,3-DPG is an intermediary metabolite in the Embden–Meyerhof glycolytic pathway in the red cells, which affects haemoglobin affinity for oxygen. Elevated concentration of 2,3-DPG decreases the affinity and thus increases the fraction of haemoglobin-bound oxygen available to the tissues, and decreased concentration of 2,3-DPG has the opposite effect[1,2]. In general, an increase in the red cell 2,3-DPG is found in response to hypoxia or anaemia and a decrease of 2,3-DPG is caused by acidosis[3,4].

Table 1 Relationship between age and concentrations of red cell 2,3-diphosphoglycerate (2,3-DPG) and haemoglobin in 'normal' population

Age (yr)	2,3-DPG* (μmol per g haemoglobin)		Haemoglobin (g per 100 ml)			
	All subjects			Males		Females
	No.	Mean±s.d.	No.	Mean±s.d.	No.	Mean±s.d.
18–24	26	14.9±1.6	36	15.0±1.0	17	13.5±0.8
25–34	35	14.4±1.3	36	15.4±0.6	8	14.0±0.9
35–44	17	14.3±1.5	14	15.6±0.6	7	13.6±1.0
45–54	17	14.2±1.5	8	14.6±0.3	9	14.1±0.9
55–64	11	14.9±1.8	6	15.1±0.3	5	14.1±1.9
65–74	56	13.8±1.9†	26	15.3±1.3	30	13.9±0.8
75–84	104	13.9±2.4†	46	14.8±0.9	58	13.9±0.8
85 and over	16	12.8±2.0†	4	15.1±0.8	12	14.1±1.1

* Regression equation for individual values of 2,3-DPG and age $y = -0.02x + 15.25$, $r = -0.2036$ ($P < 0.01$).
† Significantly lower than for Group 18–24 yr of age ($P < 0.05$, Student's *t* test).

None of the subjects were ill or seeking medical help at the time of investigation. There were no heavy smokers (more than 20 cigarettes in a day). None of the subjects were taking iron, folate or vitamin B_{12} supplements. Excluded from the study were the subjects in whom anaemia (haemoglobin concentration < 13.5 g per 100 ml for males and < 11.5 g per 100 ml for females) and/or deficiency of iron (serum iron concentration < 65 μg per 100 ml and TIBC > 410 μg per 100 ml), folate (serum and red cell folate concentrations < 4.0 ng ml^{-1} and < 160 ng ml^{-1}, respectively) or vitamin B_{12} (serum vitamin B_{12} concentration < 160 pg ml^{-1}) were found. Blood counts were determined using a Coulter Counter Model S. 2,3-DPG concentration was measured in 1:400 aqueous dilutions of heparinised or sequestrenated blood by a modified automated method of Grisolia *et al.*[14] using an Auto-Analyzer (Technicon). The concentrations of iron, folate and vitamin B_{12} in blood were determined using standard colorimetric and microbiological assays.

A total of 322 subjects (176 males and 146 females) from 18 to 97 yr of age were investigated. They were selected from the laboratory staff and the participants of the Nutritional Survey of the Elderly, carried out by the Department of Health and Social Security[5], by excluding those in whom anaemia and/or deficiency of iron, folate or vitamin B_{12} were found.

When subjects were grouped according to age a progressive decrease of 2,3-DPG concentration with advancing age was found (Table 1). The decrease from the mean 2,3-DPG of 14.9 μmol per g haemoglobin for the first group (18 to 24 yr) became statistically significant ($P < 0.05$) for the groups comprising subjects older than 65 yr. Furthermore, a significant ($P < 0.01$) inverse correlation between 2,3-DPG concentration and age was found when individual values were correlated by regression analysis. Within each group the mean 2,3-DPG concentrations in males and females were essentially the same, except in the age group 35 to 44 yr, where the difference between mean concentrations for males and females (13.6 and 15.2 μmol per g haemoglobin, respectively) was significant ($P < 0.05$). There was virtually no change in haemoglobin concentration with advancing age, and the difference in concentrations between males and females persisted in all age groups.

It is possible that the decrease of 2,3-DPG in the red cell is related to lower concentrations of plasma inorganic phosphate found in old age[6]. This is supported by observations that the red cell 2,3-DPG changes in parallel to the concentration of plasma inorganic phosphate in conditions with hyperphosphataemia[7] and hypophosphataemia[8,9]. Recently, increased concentrations of 2,3-DPG and plasma inorganic phosphate were found in children between 1 and 12 yr of age[10]. These children, however, had significantly lower than normal haemoglobin concentrations. Whether in children the 2,3-DPG concentration is increased in response to high plasma inorganic phosphate concentrations or to reduced haemoglobin is not clear.

The physiological significance of the decreased red cell 2,3-DPG in the elderly is uncertain. We calculated, on the basis of results obtained by other workers in transfused postoperative patients[11,12], that in the octogenarians studied by us the decreased 2,3-DPG would on average shift the p_{50} of the oxygen dissociation curve of haemoglobin 2 mm Hg to the left, the corresponding change in the delivery of oxygen to the tissues would only be slight. On the other hand, Birnstingl *et al.*[13] found considerably higher haemoglobin affinity for oxygen in normal men over 40 yr of age as compared to those younger than 40, when they measured oxyhaemoglobin dissociation at p_{O_2} of 9 and 13 mm Hg. Furthermore results from this laboratory indicate that in some anaemic subjects over 65 yr of age, the increase of 2,3-DPG was less than expected for the given degree of anaemia. Both latter studies would therefore indicate that at least in some elderly subjects decreased red cell 2,3-DPG may impair the mechanism of adaption to hypoxia or anaemia.

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Glucose accumulation by rat small intestine during absorption *in vivo*

GLUCOSE is transported across the mammalian small intestine even when its concentration in the lumen is lower than that in the plasma According to the hypothesis of Crane[1], this 'active transport' is coupled to the movement of sodium ions, which are maintained at a lower concentration in the mucosal epithelium of the gut than in the lumen Glucose and sodium are thought to combine with a 'carrier' molecule at some external site on the brush border of the epithelial cells, the glucose then moving uphill into the cell at the expense of the movement of sodium down its electrochemical potential gradient[2] The concentration of glucose in the mucosa rises to a level above that in the blood capillaries at the base of the epithelium, so that the sugar can move downhill into the plasma The accumulation of glucose to give a tissue concentration higher than that in the bathing fluid has repeatedly been observed in a wide variety of *in vitro* intestinal preparations and is here extended to an *in vivo* situation

About 20 cm of jejunum from male Wistar rats (200–250 g) was exposed under ether anaesthesia, and the lumen gently rinsed with 10 ml ice cold 0 9% NaCl The segment was cannulated and the lumen perfused, *in situ* (that is, with intact mesentery), with Krebs-Ringer bicarbonate medium, in a gas lift, recirculation apparatus, similar to that described by Fisher and Parsons[3] The gas mixture was 95% O_2 and 5% CO_2 During perfusion, the gut was replaced inside the body wall, and the animal maintained continuously under ether (It is likely that the relatively high blood glucose levels shown in Table 1 were due to the deleterious effects of the ether anaesthesia) After 5 min perfusion, glucose was added to the luminal circuit, and the perfusion continued for a further 10 min After this time a sample of perfusion medium was taken, the gut re-exposed, and a portion, approximately 4 cm long, immediately clamped between aluminium tongs precooled in liquid nitrogen[4] The frozen segment was kept in liquid nitrogen while approximately 1 ml blood was withdrawn from the heart into heparinised tubes

The final (that is, 15 min) concentrations of glucose in the whole gut wall (after correction for contaminating luminal and blood glucose), in the luminal fluid, and in the blood plasma, are shown, for a variety of experimental conditions in Table 1 The tissue to lumen, and tissue to plasma final concentrations ratios of glucose are also included in Table 1

When the initial concentration of glucose in the lumen was 5 55 mM, there was an approximately two-fold uphill gradient of glucose from lumen to plasma In this situation, the gut wall concentrated the glucose to a level almost 1 5 times that in the plasma, and over three times that in the lumen, so that a downhill movement of glucose could take place from the tissue into the blood Phlorizin, a specific inhibitor of glucose active transport reduced the accumulation of glucose by the tissue (tissue plasma ratio 0 90) With an initial luminal glucose concentration of 28 mM, there was a downhill gradient of glucose from lumen to plasma, so that concentration of the glucose by the tissue to a level above that in the plasma was no longer necessary, and was not observed These findings are in accord with Crane's hypothesis, but they differ from that of Esposito *et al*[7] whose technique, however, perhaps allowed loss of

Table 1 Glucose concentrations in luminal fluid, blood plasma, and gut wall

Treatment	No of observations	Lumen concentration Initial	(mM) Final	Final plasma concentration (mM)	Final tissue concentration (mM)	Tissue concentration lumen concentration	Tissue concentration plasma concentration
Control	8	5 55	4 59±0 36	11 19±1 85	15 34±2 35	3 34±0 26	1 37±0 06
Control	5	28	26 12±0 17	11 22±0 14	18 14±2 32	0 69±0 004	1 62±0 04
Phlorizin (5×10^{-5} M)	8	5 55	5 66±0 04	10 76±0 44	9 68±1 53*	1 71±0 05	0 90±0 02†
Control	4	0	ND	14 95±0 90	8 57±1 48	—	0 57±0 008
Diabetic	6	5 55	4 64±0 06	30 62±2 13	36 13±3 32	7 79±1 96	1 18±0 03

Three small (approximately 30–40 mg wt) and adjacent portions of the freeze-clamped tissue were chipped off and weighed on a torsion balance The first was ground to a paste in a ceramic mortar containing 6% (w/v) perchloric acid, the extract thawed at 4° C, centrifuged, the supernatant neutralised with 30% KOH and analysed for glucose[5] The samples of blood, and after centrifugation, plasma, withdrawn from the heart, and the luminal sample, were also deproteinised, and analysed for glucose by the same method The second tissue sample was ground with 1% (w/v) sodium citrate, to prevent blood clotting, and its absorption measured at 415 nm, since haemoglobin absorbs strongly at this wavelength A known volume of the whole blood collected from the heart was then added to the tissue extract and the increase in absorption at 415 nm used to calculate the blood volume of the frozen tissue This averaged 6 6% (in µl) of the wet weight of the tissue (in mg), which is reasonably close to published figures[6] The third portion of tissue was dried to constant weight at 110° C to determine the ratio of dry weight to wet weight of the frozen segment This was invariably less (usually around 0 16) than the true dry weight wet weight ratio of the tissue (0 215±0 0001 mean±s e) determined in separate experiments, on account of the layer of frozen medium sandwiched between the tissue layers, and could be used to calculate the amount of glucose contaminating the tissue from this source The values for tissue glucose concentration in the above table are therefore corrected for luminal glucose contamination, and for glucose contained in a space accessible to haemoglobin (The corrected values were about 30% higher than the uncorrected ones) The intracellular water associated with the tissue was assumed to be the same as the total tissue water, so that the figures for whole wall glucose concentration are probably an underestimate of the true intracellular glucose concentration Experimental diabetes was induced by the intravenous administration of streptozotocin, 65 mg kg^{-1}, 5 d before the experiment The ratio of dry weight to wet weight of frozen segments after perfusion from diabetic rats was 0 20±0 0001 and this value was used in the calculation of glucose contamination in these experiments All results are the mean ± s e m ND, Not determined

* $P < 0\ 05$ compared with control

† $P < 0\ 001$ compared with control

sugar from the mucosa during the time taken to sample the tissue

In the complete absence of glucose in the lumen, and in the presence of phlorizin (Table 1) a substantial amount of glucose was present in the gut wall probably in the mucosa (or possibly the intercellular spaces) If so, one has to postulate a backward movement of glucose from the plasma to the epithelial cells, and strong evidence in support of some form of mediated sugar movement across the basal and lateral surface of the epithelial cells has recently been reported[8-10] In the case of phlorizin inhibition, one has also to postulate some additional movement of glucose into the epithelial cells, insensitive to the action of this inhibitor

When experimental diabetes was induced by streptozotocin the plasma glucose rose to 30 mM and provided a sixfold uphill, lumen to plasma concentration gradient (Table 1) In spite of this extra load, the tissue responded by raising the intracellular glucose concentration to a level above that of the plasma, to bring about a downhill sugar gradient from tissue to plasma, suggesting that gut glucose concentrations during absorption are influenced more by the concentration of sugar in the plasma rather than that in the lumen

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In vitro cultivation of *Trypanosoma vivax* isolated from cattle

IN West Africa, *Trypanosoma vivax* is responsible for the majority of trypanosome infections in cattle and other ruminants It is not readily established in laboratory animals, and research on pathogenic isolates has been hampered by the difficulty in its establishment *in vitro* Trager[1] reported a culture method for *T vivax* using tsetse fly tissues but this medium is complex and unsuitable for metabolic studies There is therefore a need for a medium which will support the *in vitro* growth of *T vivax* and other species of trypanosomes to give reproducible results The multiplication of *T vivax*, using mammalian tissue culture media buffered with 4-(2-hydroxyethyl)-1-piperazine-ethane-sulphonic acid (HEPES) is reported here

Three isolates were used Bodija 1, Bodija 2, and Vom 36/15 The first two were obtained from blood samples collected from cattle at the Ibadan slaughterhouse and the third was supplied as a stabilate from the Nigerian Institute for Trypanosomiasis Research (NITR) Vom The two local isolates were identified as *T vivax* by morphology and failure to infect rats Heparinised cattle blood containing approximately 19×10^6 trypanosomes was inoculated intraperitoneally into sheep obtained from the Jos Plateau, a tsetse-free area The stabilate, Vom 36/15 was injected into a sheep The parasitaemia, general health and haematology of the sheep were monitored daily

Trypanosomes for cultivation studies were isolated from whole blood by centrifugation, DEAE cellulose columns[2], or erythrocyte lysis[3] followed by DEAE cellulose columns The last method was found to be the most efficient, important with the low parasitaemias frequently associated with *T vivax*

Isolated trypanosomes were washed twice and resuspended in Hanks saline +5% foetal calf serum, and inoculated into culture media Some of the media and additives tried are included in Table 1 The number of trypanosomes inoculated into each ml of culture was $0\ 48 \times 10^6$ or $1\ 0 \times 10^6$ The number in culture at various times after inoculation was determined by dilution in a fixative-staining solution (0 5% toluidine blue, 1% sodium citrate, 10% formalin, in distilled water) and counting in a haemocytometer When trypanosomes numbered less than $0\ 03 \times 10^6$ ml^{-1} survival was monitored by evidence with phase microscopy of freely motile trypanosomes At each sampling, Giemsa stained smears were prepared after centrifugation and resuspending the trypanosomes in serum NADH diaphorase technique was used to demonstrate mitochondria[4]

Plasma from infected and uninfected animals was stored at −10° C before incorporation into culture media

As indicated in Fig 1, cultures of *T vivax* (Bodija-1 isolate) were made at parasitaemias of 11, 14, 17, 18, and 22 d after infection of sheep Table 1 presents the results of the cultures of the 22-d isolate of Bodija 1 Cultured in Medium 199 buffered by HEPES (Medium 199H) with foetal calf serum and homologous plasma (prepared the same day as the culture), and incubated at 25° C, organisms increased fourfold within 24 h *T vivax* from the same day isolated in Medium 199H and either γ-globulin free serum or foetal calf serum, increased in numbers 3 8 and 3 1-fold respectively within 24 h After the initial increase, numbers decreased at 48 h (1 95 to 1 55 ×

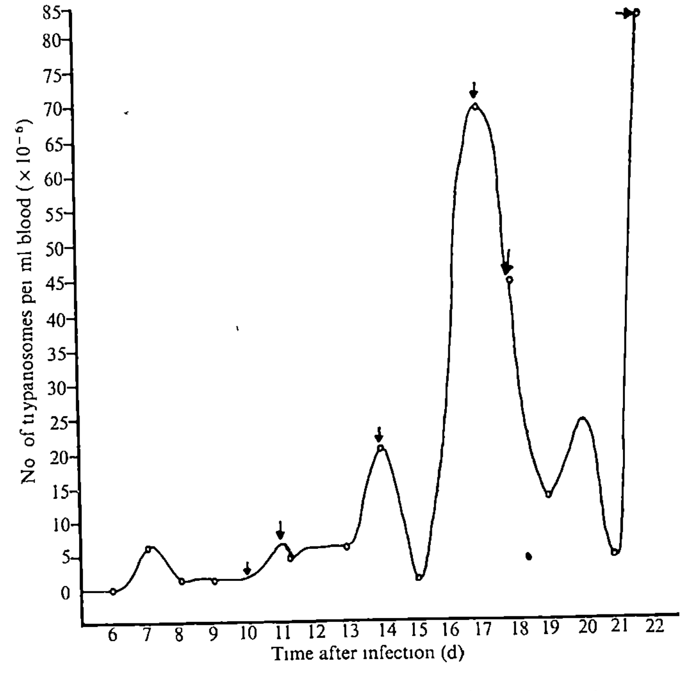

Fig 1 Parasitaemia of *T vivax* (Bodija 1) in sheep Arrows indicate points in parasitaemia where cultures of *T vivax* were prepared

Table 1 Growth of *T. vivax* (Bodija 1) at 22 d after infection of a sheep, in various media at 25° C

Medium	No. at 0 h (×10^6 ml^{-1})	No. at 24 h (×10^6 ml^{-1})	Increase ratio (No. at 24 h, No. at 0 h)
199H*, foetal calf serum†, homologous plasma‡	0.48	1.95	4.0
199H, γ-globulin free serum†	0.48	1.86	3.80
199H, foetal calf serum	0.48	1.50	3.10
199H, foetal calf serum, homologous plasma§	0.48	0.66	1.30
Modified Grace's insect medium, haemolymph†, homologous plasma‡	0.48	0.54	1.10
Modified Grace's insect medium, lactalbumin hydrolysate‖	0.48	0.54	1.10
199H, foetal calf serum erythrocyte lysate§^{5}	0.48	0.00	0.00
199H, foetal calf serum, whole blood filtrate^6	0.48	0.00	0.00

* Medium 199 with Hanks saline and HEPES buffer.

† Grand Island Biological Co., Grand Island, New York.

‡ Plasma from the infected sheep from which blood was collected for this culture, on day 22 of infection.

§ Plasma from the infected sheep from which blood was collected for this culture, on day 18 of infection.

‖ Sigma, St Louis, Montana.

10^6 ml^{-1} and 1.5 to 1.05 × 10^6 ml^{-1} for example). From day 2 the number of trypanosomes continued to decrease. Throughout the period *in vitro*, however, but more noticeably after subculture every 72 h, there were groups of trypanosomes which appeared to be the result of division (early rosettes, later clumps). With Giemsa stained smears, forms which appeared to be dividing were present. Motile trypanosomes were present after 10 d *in vitro* when the cultures were terminated. In Trager's work as well as in long term cultures of primary lines with other species of trypanosomes, initial growth may be followed by a decrease or near disappearance of the line, later resumption of growth occurs from a very few competent organisms. Other media were less successful in supporting the growth of *T. vivax* (Table 1).

The populations of *T. vivax* isolated in Medium 199H and foetal calf serum at 25° C on days 11, 14, 17, and 18 after infection, survived 24, 96, 24, and 72 h, respectively.

The morphology of *T. vivax in vitro* during the growth period is similar to that of the forms seen in the whole blood when collected from the host, except that by 4 h *in vitro*, the kinetoplast moved 1–2 μm anteriorly. The mitochondrion as demonstrated by the NADH diaphorase technique did not seem to be significantly altered during the first 24 h period of growth *in vitro*.

Medium 199H with foetal calf serum and homologous plasma also supported growth at 25° C or 31° C of selected isolates of Bodija 2 and Vom 36/15.

Infectivity studies in ruminants were not carried out in this study as the volume of cultures was too small.

Our data indicate that trypanosomes taken at different times during the course of infection with *T. vivax* behaved differently in identical *in vitro* conditions. *T. vivax* is generally considered monomorphic, although evidence of morphological variation between strains has been reviewed recently[7]. We have observed considerable physiological pleomorphism within strains of *T. vivax*.

The optimum *p*H for the growth of pathogenic trypanosomes is considered to be 7.4. HEPES buffer[8] which has an efficient buffering capacity in the *p*H range 7.4–7.5 (*p*K 7.31 at 37° C and a *p*K$_a$ of 0.014) may therefore promote the multiplication of *T. vivax*. The use of homologous plasma seems to enhance growth.

Our method may be useful for the short term cultivation of *T. vivax* for metabolic, biochemical, immunological and pharmacological studies.

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Neuraminidase treatment of adrenal cells increases their response to cholera enterotoxin

A PRIMARY action of cholera enterotoxin is to increase adenyl cyclase activity in the intestine and the elevated levels of cyclic AMP are thought to be responsible for the water loss and dehydration associated with cholera[1–4]. Enterotoxin also stimulates a variety of other tissues including cultured adrenal tumour cells[5,6]. In these instances the actions of the toxin are very similar to those hormones whose effects are mediated through regulation of intracellular cyclic AMP in their target tissues. A common characterisic of the hormonal responses, however, is that cyclic AMP levels are usually raised within a few seconds whereas the response to enterotoxin, in all the tissues tested, is always accompanied by a lag period of at least several minutes. We have shown previously that pretreatment of isolated rat adrenal cells with neuraminidase reduces the response to ACTH indicating that membrane sialic acid may be involved in stimulation of adenyl cyclase by this hormone[7,17]. We report here that in sharp contrast to the results obtained with ACTH, the response of adrenal cells to enterotoxin is markedly enhanced by treatment with neuraminidase.

Isolated rat adrenal cells prepared by collagenase-trypsin digestion[7] respond to enterotoxin with a characteristic lag period of between 30–45 min. Subsequently the rates of both cyclic AMP and corticosterone production are linear for at least 1 h. When the cells are treated with 20 mU ml^{-1} neuraminidase in conditions shown to liberate membrane sialic acid[7,17], the response to enterotoxin is increased. The results in Table 1 indicate that in neuraminidase treated cells, the production of both corticosterone and cyclic AMP is increased at all tested concentrations of enterotoxin, the increase is relatively greater for cyclic AMP than it is for corticosterone and the dose of enterotoxin required to produce maximum stimulation is similar for both corticosterone and cyclic AMP.

Figure 1 shows the time course of corticosterone synthesis by control and neuraminidase-treated cells in the presence of maximally stimulatory levels of enterotoxin. The maxi-

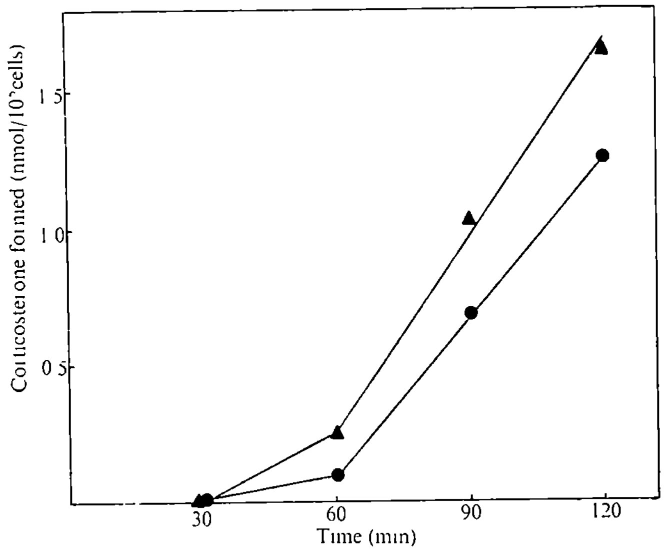

Fig 1 Time course of corticosterone synthesis by control (●) and neuraminidase-treated (▲) cells in response to enterotoxin In the absence of enterotoxin, corticosterone production was negligible (<0 05 nmol per 10^5 cells per 2 h) in both groups Concentration of enterotoxin was 1 μg ml^{-1}

mum rates for corticosterone production, attained after 60 min of incubation, are 1 17 and 1 45 nmol per 10^5 cells per h for control and neuraminidase-treated cells respectively Extrapolation of these curves indicates that the lag period is shortened by about 6–7 min in the neuraminidase-treated cells Thus, the increased response of neuraminidase-treated cells to enterotoxin may be the result of a combination of two effects first, the increased rate of synthesis and, second, the shortening of the lag period

It is not yet clear whether neuraminidase treatment simply 'unmasks' more enterotoxin receptors on the cell membrane or produces a change in the configuration of receptors Recent studies have indicated that the biological receptor for enterotoxin may be a ganglioside[8–12] King and van Heyningen[10] have shown that addition of neuraminidase-resistant monosialoylganglioside (G_{M1}) with enterotoxin can prevent the stimulation of intestinal adenyl cyclase by the latter Such an effect was not observed with neuraminidase-sensitive di- and trisialosylgangliosides The treatment of these gangliosides with neuraminidase, however, rendered them capable of abolishing the effect of enterotoxin—presumably by converting them to G_{M1} Cuatrecasas[11] has shown that among all the gangliosides tested, G_{M1} was the most effective in inhibiting the binding of enterotoxin to liver membranes In the same study it was found that digestion of liver membranes with neuraminidase resulted in a slight enhancement in the ability of the membranes to bind enterotoxin Thus, it is possible that increased sensitivity of the neuraminidase-treated cells to enterotoxin in our study was due to the conversion of complex di- and trisialosylgangliosides to monosialosylganglioside in the intact cell membrane Our studies also provide a plausible explanation for the presence and function of large amounts of neuraminidase found in *Vibrio cholerae*

Table 1 Effect of neuraminidase (Nase) treatment of adrenal cells on the formation of cyclic AMP and corticosterone in response to enterotoxin

Toxin ($ng\ ml^{-1}$)	Corticosterone formed nmol per 10^5 cells per 2 h Control cells	Corticosterone formed nmol per 10^5 cells per 2 h Nase treated cells	Cyclic AMP formed pmol per 10^5 cells per 2 h Control cells	Cyclic AMP formed pmol per 10^5 cells per 2 h Nase treated cells
0	<0 05	<0 05	0 5	1 4
100	0 54	1 53	2 7	13 7
300	1 28	2 00	4 9	33 9
1,000	1 99	2 63	24 6	48 5
3,000	1 91	2 53	24 4	48 8

Procedures for the preparation of isolated adrenal cells[7 13] and neuraminidase treatment[7 17] have been described previously Cyclic AMP was measured by the protein binding method[14 15] and corticosterone by sulphuric acid-fluorescence[16]

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Prostaglandin E_1 action on sinus pacemaker and adenylyl cyclase in kitten myocardium

A QUANTUM mechanical analysis of Kier *et al*[1,2] indicates that the amine group of the catecholamine isoprenaline may not be essential to produce pharmacological effects mediated through adrenergic β receptors Based on this and on conformational similarities between the ring, β-hydroxyl group and alkyl areas of N-*n*-propylnoradrenaline and prostaglandin (PG) E_1, these authors predicted that PGE_1 should be able to interact positively with adrenergic β receptors Isolated tissues from kitten heart are appropriate systems to investigate effects of drugs which are β-receptor dependent and we tested the predictions of Kier *et al* by investigating if PGE_1 produced positive inotropic and chronotropic effects and whether these effects are transmitted through the adrenergic β receptors

It is a general finding that dose response curves of positive inotropic and chronotropic effects of catecholamines are similar when obtained with driven left atrial strips and with

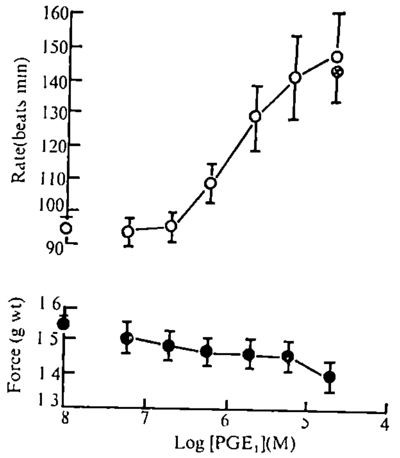

Fig 1 Nonadrenergic positive chronotropic effect of prostaglandin PGE_1 Tissues were from kittens (weight 350–800 g, either sex), pretreated with reserpine (5 mg kg^{-1} subcutaneously, 24 h) Cumulative concentration effect curves of PGE_1 on spontaneously beating right atria (○) and on left atrial strips driven at 2 s intervals with barely suprathreshold pulses of 5 ms duration (●) Values are mean ± s e m from four pairs of left and right atria in the same 50 ml organ bath containing gassed (95% O_2, 5% CO_2), warm (32 5° C) modified Krebs solution[3] 6×10^{-7} M (±)-propranolol was added for 65 min after 2×10^{-5} M PGE_1 had produced an equilibrium effect on right atria (±)-Propranolol did not influence the effect of PGE_1 (cross in circle) Open and closed circles on ordinate are values of basal rate and force, respectively, before PGE_1 was administered

spontaneously beating right atria[3] We found that PGE_1 selectively produced positive chronotropic effects but not positive inotropic effects (Fig 1) Similarly, on six driven papillary muscles (5 s intervals, 32 5° C, from kittens pretreated with reserpine), concentrations from 2×10^{-8} M to 4×10^{-5} M PGE_1 did not modify the strength of isometric contraction or the rates of contractions and relaxation This dissociation of positive chronotropic effects and lack of inotropic effects of PGE_1 is not characteristic for adrenergic agonists

To see whether the selective positive chronotropic effect of PGE_1 on right atria was mediated by adrenergic β receptors, β-receptor blocking agents were used Thus, if PGE_1 is a β agonist its activity should be antagonised by conventional β-blockers and greater concentrations of the prostaglandin should surmount the blockade allowing the estimation of an apparent dissociation equilibrium constant (K_B) for the blocker–receptor interaction If the blocker and the prostaglandin interact both with the same receptor this K_B should not differ from the K_B estimated from the antagonism of the blocker against a classical β agonist such as isoprenaline With this in mind, we first added 6×10^{-7}M (±)-propranolol to the atria under the influence of PGE_1 (±)-Propranolol did not modify the positive chronotropic effect of PGE_1 on four right atria (Fig 1) In two of these atria 1×10^{-7}M (−)-KL 255 was administered 65 min after (±)-propranolol and an additional 30 min incubation of (±)-propranolol plus KL 255 did not influence the positive chronotropic effect of PGE_1 Because the employed concentrations of both (±)-propranolol and (−)-KL 255 were at least 200 times greater than their respective K_Bs[3,4] they produced at least 99 5% β-receptor occupancy Since the positive chronotropic effect of PGE_1 persisted under these conditions, it must be ruled out that it is mediated through adrenergic β receptors[1,2,5]

PGE_1 presumably modifies the ion permeability of the membrane of sinus pacemaker cells in such a way that steeper prepotentials and tachycardia result If so, it may perhaps be related to an increase of adenylyl cyclase activity in the membrane of pacemaker cells and concomitant increase of intracellular cyclic 3′,5′-adenosine monophosphate (cyclic AMP) levels Based on the previous findings, activation of pacemaker cell adenylyl cyclase by PGE_1 should be independent of β-receptor action We therefore explored the possible effect of PGE_1 and isoprenaline on adenylyl cyclase activity in membranes of kitten atria In such membranes PGE_1 increases adenylyl cyclase activity by 50% (Fig 2) As expected, this effect of PGE_1 is unaffected by 10^{-7}M (±)-propranolol, indicating that it is not mediated by adrenergic β receptors To check whether these membranes preserved their β receptors, the action of (−)-isoprenaline was investigated (−)-Isoprenaline increased adenylyl cyclase activity about three times more than PGE_1 (±)-Propranolol (10^{-7}M) surmountably antagonised the action of (−)-isoprenaline (Fig 2)

We calculated an apparent K_B of about 3×10^{-9}M for (±)-propranolol from the concentration ratio of concentration-effect curves of (−)-isoprenaline obtained in the absence and the presence of the blocker according to equation K_B = (±)-propranolol/(concentration ratio — 1) This value of K_B for (±)-propranolol is the same as the K_B between the β blocker and receptors mediating positive inotropic and chronotropic responses of (−)-isoprenalaine[4], indicating that the β receptors are unchanged in cell-free membrane particles[6]

Concentration effect curves to PGE_1 on both intact right atria—positive chronotropic effect, and on atrial membrane particles—stimulation of adenylyl cyclase, seem to correlate This correlation does not however necessarily imply functional dependency If the enhanced adenylyl cyclase activity is a specific step between a hypothetical PGE_1 receptor and a site on the pacemaker cell where permeability changes lead to increased chronotropism, one would expect adenylyl cyclase in membrane particles from the ventricle not to respond to PGE_1 But PGE_1 was found to increase also the adenylyl cyclase activity in membranes from ventricular myocardium (Fig 3) This effect is only about 2/5 of the maximum increase of activity obtained with (−)-isoprenaline As in atrial membranes, the effect of PGE_1 is not modified by 1×10^{-7}M (±)-pro-

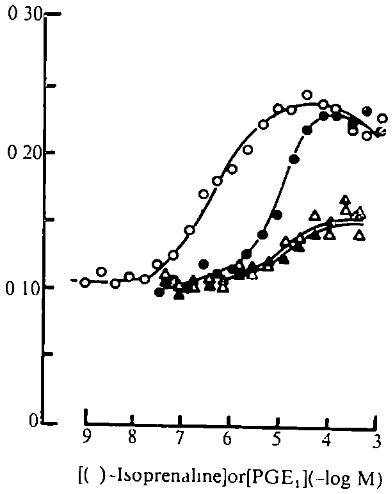

Fig 2 Nonadrenergic and adrenergic adenylyl cyclase stimulation by PGE_1 and (−)-isoprenaline, respectively 10,000*g* membrane particles of atria from kittens pretreated with reserpine were prepared as described elsewhere[12] Particles (78 µg protein) were preincubated in 20 µl of 0 5 mM $KHCO_3$, 2 5 mM EGTA, 25 mM Tris-HCl, *p*H 7 5, at 4° C, during 120 min with (solid symbols) or without (open symbols) 10^{-7} M (±)-propranolol, later 10 µl of 1 mM $KHCO_3$, 0 05 mM EGTA, *p*H 7 5, with or without (−)-isoprenaline (circles) or PGE_1 (triangles) were added and the mixture further preincubated for 90 min at 4° C Incubation was initiated by adding 20 µl containing ingredients to give in a final volume of 50 µl 2 0 mM α-^{32}P-ATP (1 5 × 10^6 c p m), 3 5 mM $MgCl_2$, 1 0 mM cyclic AMP, 20 mM creatine phosphate, 0 2 mg ml^{-1} creatine kinase and 25 mM Tris-HCl, *p*H 7 5 The incubation lasted 10 min at 32 5° C, it was stopped with 100 µl of 40 mM unlabelled ATP, 12 5 mM ^{3}H-cyclic AMP (about 10,000 c p m) and 1% sodium dodecyl sulphate followed by immediate boiling for 3 5 min The ^{32}P-cyclic AMP formed was determined by the method of Krishna *et al*[13]

pranolol and seems therefore not to be mediated by adrenergic β receptors In contrast, (±)-propranolol surmountably shifted the concentration-effect curve of (−)-isoprenaline towards greater amine concentrations (Fig 3) which allowed us to estimate a K_B of about 4 × 10^{-9}M for (±)-propranolol This K_B is the same as the K_B between (±)-propranolol and β receptors mediating inotropic effects in papillary muscles of kittens, indicating that the β receptors are preserved in ventricular membrane particles[6] The similarity of K_Bs of (±)-propranolol on membrane particles from atria and ventricles is evidence that the membranes from both tissues have similar β receptors

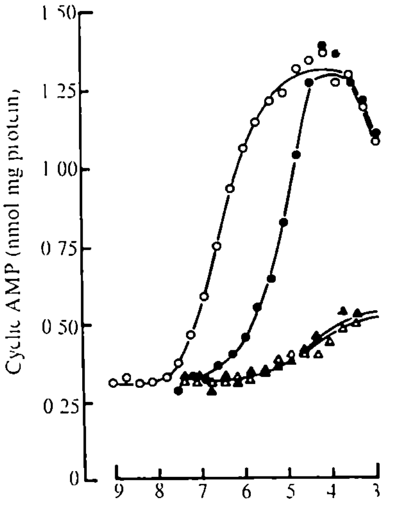

Fig 3 Comparison of adenylyl cyclase stimulation by PGE_1 (triangles) and by (−)-isoprenaline (circles) on membrane particles of ventricle For incubation medium and other conditions, see legend to Fig 2 Solid symbols with 10^{-7} M (±)-propranolol, open symbols without (±)-propranolol

Our results support the suggestion that PGE_1 would increase chronotropism through an increase of adenylyl cyclase activity in cardiac membranes Cyclic AMP has been shown to increase activity of pacemaker cells not only in atria, but also in Purkinje fibres The increase of heart cyclic AMP[8], and membrane adenylyl cyclase activity to PGE_1 seems to be of no consequence for inotropism and if PGE_1 promotes chronotropism through stimulation of adenylate cyclase activity, this effect is either restricted to intact pacemaker cells or does not affect mechanisms that lead to increased strength of contraction of myocardium The fact that PGE_1 stimulates adenylyl cyclase and chronotropism through receptors which are not adrenergic β receptors does not rule out the possibility that catecholamines perhaps catalyse the synthesis of endogenous prostaglandins This has been suggested for the action of cyclase stimulating hormones in the ovary[9], the thyroid[10], and stomach muscle [11]

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Effect of foetal calf serum and epidermal growth factor on DNA synthesis in explants of chick embryo epidermis

THE epidermal growth factor (EGF) is a peptide hormone which has been isolated from the submaxillary glands of male mice[1,2] It enhances proliferation and keratinisation of embryonic and neonatal epidermis *in vivo* and *in vitro*[3,4] In spite of these morphologically well documented effects, attempts to demonstrate stimulation of epidermal DNA synthesis have failed[5] In this communication we report that EGF considerably enhances precursor incorporation into DNA of chick embryo epidermal explants and that the responsiveness of the tissue to the hormone is increased by foetal calf serum

Explants of epidermis of 9-d-old chick embryos were grown in Eagle's minimal essential medium in the presence of 15% foetal calf serum By means of pulse-labelling with tritiated thymidine a uniform rate of DNA synthesis was observed over the first 7 d of cultivation The DNA labelling was decreased if serum was omitted, but EGF strongly stimulated serum-free cultures (Table 1)

In the following experiments the explants were preincubated for one day before addition of the hormone Fig 1*a* shows the time course of the stimulatory effect of EGF on the labelling of DNA in the total absence of serum Maximal stimulation was observed with 2 μg ml^{-1} EGF whereas higher doses led to a decrease (Figs 1 and 2) When the explants were precultivated in the presence of 15% foetal calf serum for one day, the control level of DNA synthesis (without EGF) was found to be approximately twice as high as that achieved without serum (Fig 1*b*) Under these conditions an EGF dose as low as 0 02 μg ml^{-1} (3×10^{-9} M) was sufficient to induce the maximal response (Fig 1*b*) With higher concentrations an inhibition of DNA labelling was observed (Figs 1*b* and 2)

The response of the tissue to the hormone occurred after a certain lag phase which depended on the EGF dose In the absence of serum (Fig 1*a*) the lag phase was much longer than in serum-containing cultures where it was less than 2h, the time of the first measurement (Fig 1*b*) In order to decide whether the serum factor was identical with EGF, the explants were precultivated with 2 μg ml^{-1} EGF instead of serum One day later a further 2 μg ml^{-1} EGF were added and the incubation was continued for 24 h Marked labelling of the DNA was found (specific radioactivity of 1,880 ± 143 c p m $μg^{-1}$ in ten experiments) compared to a control in which the second addition of EGF was omitted (227 ± 42 c p m $μg^{-1}$) This is in clear contrast to the inhibition of DNA labelling by 2 μg ml^{-1} EGF in cultures preincubated with serum instead of EGF (Fig 1*b*) The serum effect is thus obviously not due to contamination by EGF

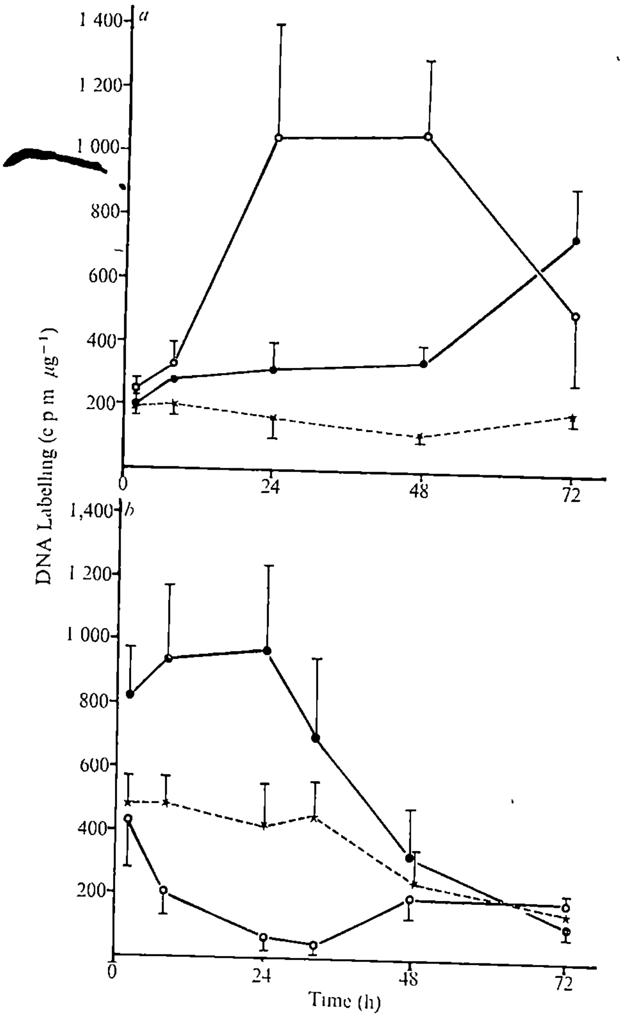

Fig 1 Effect of epidermal growth factor (EGF) on the pulse-labelling of DNA in chick embryo epidermal explants cultivated in the absence (*a*) or presence (*b*) of serum EGF was prepared from frozen submaxillary glands of male mice according to ref 2 The explants were dissected from the back-skin of 9-d-old chick embryos (White Leghorn) and epidermal sheets were separated from connective tissue by incubation with 0 1% collagenase (Boehringer Mannheim) in Dulbecco's phosphate-buffered saline (PBS) at 37° C for 25 min[4] The method used for tissue culture was based on that described by Cohen[4] Intact sheets of epidermis were layered on to pieces (0 5 × 0 5 cm) of Millipore filter (Solvinert, pore size 0 25 μm) with the peridermal side facing upwards The filters were placed on agar platforms[4] and incubated in plastic Petri dishes (Falcon, 3 5 cm diameter) with 1 ml Eagle's minimal essential medium (MEM) at 37° C in the presence of 5% CO_2 Each Petri dish contained 3 explants For DNA labelling three filters with explants were removed from the agar platforms, placed into an Eppendorf reaction vial containing 5 μCi ^{3}H-methyl thymidine (NEN, Boston, USA) in 1 ml medium and incubated at 37° C for 1 h The medium was then replaced by 1 ml ice-cold 0 4 M perchloric acid and the samples were allowed to stand in an ice-bath for 10 min After washing with 1 ml ethanol (twice) and 1 ml ether (twice) as well as with 1 ml ice-cold 0 4 M perchloric acid each, each filter was transferred into 0 4 ml 0 3 M NaOH and incubated at 30° C for 90 min During this procedure the epidermal sheet was solubilised The filter was removed and the solution was acidified with 0 1 ml 3 M perchloric acid After 60 min (0–4° C) the precipitate was collected by centrifugation, washed three times with 1 ml 0 4 M perchloric acid and finally dispersed in 0 3–0 4 ml 0 5 M perchloric acid and heated at 95° C for 20 min Aliquots of the clear supernatant were used for measuring radioactivity and DNA content[6] The explants were preincubated in MEM (without serum) for 24 h before the addition of EGF ★ controls (without EGF), ● 0 02 μg ml⁻¹ EGF, ○ 2 μg ml⁻¹ EGF Each point represents the mean of > 10 determinations (± s d)

Table 1 Pulse-labelling of DNA in chick embryo epidermal explants after 24 h cultivation in the absence and in the presence of foetal calf serum (15%) or epidermal growth factor

EGF (μg ml⁻¹)	Serum	Specific radioactivity of DNA (c p m μg⁻¹)
–	+	740 ± 240
–	–	348 ± 172
0 2	–	1294 ± 245
2	–	2830 ± 573

For experimental details see Fig 1 (± s d , *n* > 10)

These results show that EGF strongly induces DNA synthesis in explants of foetal epidermis and that foetal calf serum increases the tissue's responsiveness to the hormone approximately one hundredfold Since similar observations could not be made with epidermis of adult animals the stimulatory effect of EGF is apparently restricted to the foetal or embryonic tissue On the other hand, the healing of wounded corneal epithelium of adult rabbits is accelerated by the factor, although as soon as the regeneration is completed, the effect disappears completely[9]

We have recently proposed[7] that the proliferative compartment of foetal epidermis is built up by 'pluripotential' stem cells which are replaced by 'committed' stem cells during adolescence and that epidermal wound healing in adult animals is accompanied by a dedifferentiation of committed, or by an activation of residual 'pluripotential' stem cells (refs 10 and 11 and unpublished data)

Whereas a committed cell population may be considered to be a self-regulated system, in a pluripotential cell population the equilibrium between growth and specific tissue function must necessarily be controlled by exogenous factors Since EGF has been shown to induce cell proliferation as well as tissue function in interfollicular foetal epidermis[3,4], it may be such a factor

At present it is impossible to tell whether the primary activity of EGF is to induce growth or keratinisation, or whether it does both by controlling tissue homeostasis If EGF induces keratinisation and the stimulation of growth is due to compensatory proliferation of the remaining stem cells, the inhibitory effect of overdoses of EGF (see also ref 8) could be explained as the consequence of a rate of keratinisation too high for compensatory proliferation, so that the stem cell pool runs empty

The ontogeny of epidermis may include several stages of commitment One of the first might be the induction by specific

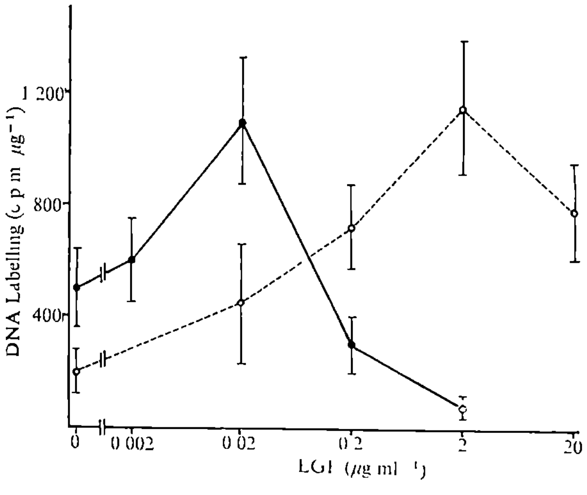

Fig 2 Dose-response relationship of the effect of EGF on pulse-labelling of DNA in chick embryo epidermal explants EGF was added after preincubation of the explants for 24 h The DNA was pulse-labelled after 48 h The experiment was done either in the absence (○) or in the presence (●) of 15% foetal calf serum Each point represents the mean of > 10 determinations (± s d) For details see Fig 1

serum factors of responsiveness to hormones capable of controlling tissue homeostasis, such as EGF. This stage, during which tissue function depends entirely on exogenous stimulation, is replaced by a second stage where the tissue's own regulation, such as the chalone mechanism[6-11], is established.

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Heterogeneity of carcinoembryonic antigen and its fractionation by con A affinity chromatography

THE term carcinoembryonic antigen (CEA) was first used by Gold and Freedman to describe a tumour-associated antigen found in the cellular membrane of adenocarcinomas from endodermally derived digestive system epithelia[1]. It is conveniently prepared from liver metastases of primary colonic tumours by initial extraction with perchloric acid followed by Sepharose 4B and Sephadex G-200 chromatography[2,3]. Initial optimism about the specific occurrence of CEA in digestive tract tumours and in the serum of patients with such lesions has not been sustained and CEA is now known to occur in both normal colonic mucosa and other normal tissues[4] and in the sera of normal individuals, although in relatively low amounts[5]. Elevated levels of CEA have been found in a wide variety of neoplastic diseases and also in non-neoplastic disorders such as gastrointestinal polyps, alcohol cirrhosis, uraemia and inflammatory bowel disease[6-8].

It seems possible that the inadequate specificity of CEA radioimmunoassay, as used to detect certain types of human cancer, might result from heterogeneity in the CEA preparation used in the assay and to raise antisera. An associated glycoprotein CEX[9], separated from some batches of CEA by gel filtration, appears to be immunologically identical to NGP[10] and NCA[11] antigens originally reported. Other workers, however, using a variety of techniques[3,9,12,13], have demonstrated the heterogenous nature of CEA which had been separated from CEX. Charge heterogeneity and variation in electrophoretic mobility may reflect variability in sialic acid content although residual heterogeneity of CEA is still seen after neuraminidase treatment. Also, different batches of CEA are known to vary in terms of carbohydrate/protein ratio and their amino acid constitution and it is significant that the most marked variation occurs between preparations from tumours of different tissues[14].

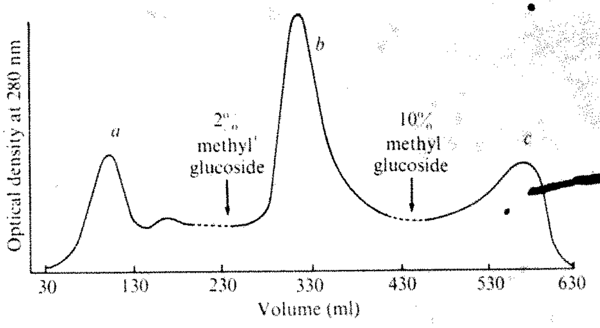

Fig. 1 Con A–Sepharose chromatography of CEA. 30 ml of purified CEA solution[2] containing 98 mg of CEA was dialysed against 0.1 M sodium acetate buffer (*p*H 6) containing 1 M NaCl, 10^{-3} M $CaCl_2$, 10^{-3} M $MgCl_2$ and 10^{-3} M $MnCl_2$. The solution was then applied to a column of con A–Sepharose (Pharmacia), bed volume 66 ml and eluted with the above buffer. After the unbound fraction 1 was collected, a 2% solution of methyl glucoside in the above buffer was applied and fraction 2 collected. The third fraction was eluted using 10% methyl glucoside solution. The column was finally washed with 20% methyl mannoside before reuse. The recovery of protein in each fraction, determined by optical density at 280 nm was 10%, 50% and 20% respectively.

Here we describe the use of con A immobilised on Sepharose 4B (ref. 15) to separate CEA into immunologically active fractions. Of particular relevance to our work are the results of Chu *et al.*[16] who have shown that CEA can be specifically bound to con A and the binding reversed by another saccharide. CEA used in the present study (M-12) was prepared by the method of Krupey *et al.*[2] from a large liver metastasis and separated on a column of con A–Sepharose into three fractions as illustrated in Fig. 1. To demonstrate that fraction 1 did not contain some fraction 2 because of overloading of the column, fraction 1 was reapplied to a new column; it was not absorbed and no detectable peak was eluted with 10% methyl glucoside. Each fraction was concentrated to approximately 1 mg ml^{-1} protein by ultrafiltration using an Amicon PM 10 membrane and tested by double diffusion against various anti-CEA antisera.

The preparation and comparison of the antisera formed the major part of this work. Antisera were prepared in rabbits by injecting 200 µg of a purified batch of CEA[2] in complete Freund's adjuvant, intradermally in the flank; a booster was given 40 d later. Antiserum 174, after extensive absorption with pooled normal colonic mucosa extract, normal human plasma and liver extract, produced a reaction of identity in double diffusion with fractions 1, 2 and 3 and an additional reaction with fraction 2 (Fig. 2*a*). The common antigenic determinant has been designated group A whereas the additional determinant in fraction 2 has been designated group B. Similar results have been obtained with a later CEA preparation (M-31). Anti-NCA (ref. 11) and anti-CEX (ref. 9) antisera on the other hand failed to react with fractions 1 and 2 but gave a strong reaction with fraction 3 identical to the reaction given by NCA previously prepared from a crude CEA extract (Fig. 2*b*).

These results are in contrast to those obtained with antiserum 224. After absorption, a sharp precipitin line was produced with fraction 1 but no reaction was given with fractions 2 and 3 (Fig. 2*c*). The determinant with which antiserum 224 reacts is quite distinct from the A group determinant (Fig. 2*d*), and has been tentatively designated the Y group, although it is not yet clear whether this is a widely occurring CEA component.

Various antisera, such as 'ace' 10, 22, 24 and 36, reacted similarly to antiserum 174 and gave a single line of identity between all three fractions. With the A-group specific line given by the latter antiserum (Figs 3*a* and 3*b*) 'ace' 24 antiserum gave an additional line with fraction 3, (cross-reacting with the A-group specific line), which has been shown to be identical with the line given by anti-CEX antiserum and the associated glycoprotein CEX. In addition, 'ace' 36 antiserum produces an additional line against fraction 2 (Fig. 3*c*) which is identical to the B group-specific line given by antiserum 174. It is not established whether the B group reported here is the same as the unique CEA determinant recently reported by Tomita *et al.*[17], or alternatively the β-E protein reported by Orjasaeter *et al.*[18] which was shown not to be identical to Gold's original CEA.

To exclude the possibility that our antisera may be reacting ~~strongly~~ with components in normal liver and colon, even after extensive absorption, we have tested the antisera against preparations containing up to 50 mg ml^{-1} of freeze-dried perchloric acid extract in double diffusion studies. Antiserum 224 and the B-group activity of antiserum 174 failed to produce a precipitin line in any case. Anti-NCA antiserum, however, produced a strong line with the extracts showing the presence of the associated glycoprotein. In addition, strong lines were given between anti-NCA antiserum and extracts (20 mg ml^{-1}) of normal lung and spleen in agreement with the original reports of Mach and Pasztaszeri[9] and von Kleist *et al.*[11]. Using antiserum 174 and the monospecific anti-CEA antisera of Todd (which react with the A-group) we have been able to demonstrate the presence of CEA in some normal colon and lung tissue extracts at concentrations of about 50 mg ml^{-1}. (Fig. 3*d*). The failure to detect both B and Y-group antigens on the other hand may be significant, although pending quantitative estimation by radioimmunoassay, their 'normal' level relative to that of CEA as measured by most anti-CEA antisera, is unknown.

Our results, reported here in preliminary form, show that CEA can be separated into at least two immunologically related fractions in addition to its separation from the well documented NCA. Also, we have shown that CEA fractions 1 and 2 contain determinants (which may be on separate molecules) which can be distinguished by appropriate antisera. These fractions, however, remain indistinguishable by anti-CEA antisera such as the Todd 'ace' antisera which appear to be reacting with a group common to each of the fractions.

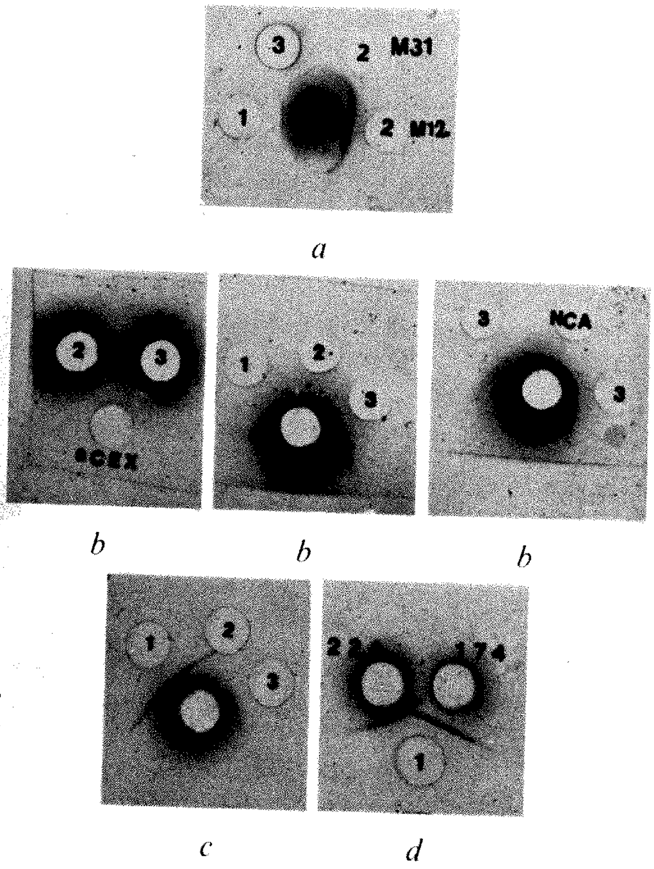

Fig. 2 *a*, Ouchterlony double-diffusion reactions between antiserum 174 (centre well) and fractions 1, 2 and 3 showing line of identity and an additional line produced by fraction 2 from CEA preparations M-12 and M-31. *b*, Reaction of anti-NCA and anti-CEX antisera (bottom wells) with fraction 3 and NCA showing an identical reaction. *c*, Reaction of antiserum 224 (centre well) with fraction 1 and, *d*, reaction showing dissimilar reactions of antisera 224 and 174 against fraction 1.

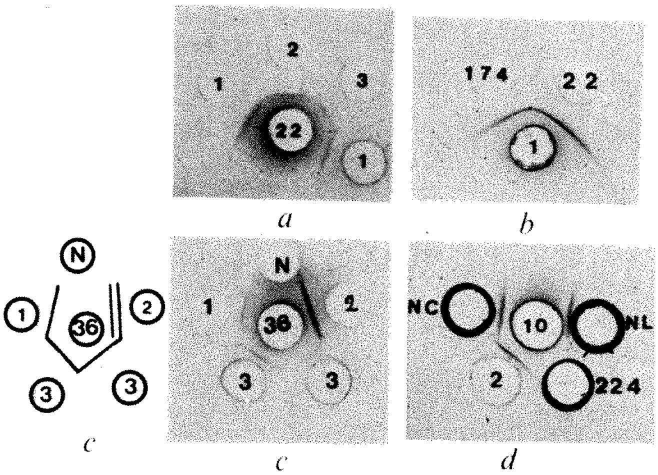

Fig. 3 *a*, Reaction of 'ace' 22 antiserum (centre well) with fractions 1, 2 and 3 showing line of identity. *b*, Reaction of identity between antiserum 174 and 'ace' 22 antiserum against fraction 1. *c*, Reaction showing additional line given by 'ace' 36 antiserum and fraction 2. *d*, Reaction using 'ace' 10 antiserum (centre well) to show the presence of CEA in normal colon (NC) and normal lung (NL); a line of identity with fraction 2 is obtained. Antiserum 224 fails to react.

The immunological differentiation between the con A bound and unbound CEA's raises fundamental questions about the chemical nature of CEA. It is necessary to know whether the A and Y and also the A and B groups are situated on different CEA-like molecules and whether further fractionation is possible. It is also important to know how sialic acid is distributed between the CEA fractions and how its removal will affect the binding properties of CEA to con A. In addition to these basic problems, further fractionation of CEA preparations may afford reagents for improving the specificity of the CEA assay and indicate whether circulating CEA shows a similar heterogeneity to preparations isolated from tumour tissue.

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Combined hyperthermia and radiation suggest an alternative to heavy particle therapy for reduced oxygen enhancement ratios

THE American Cancer Society estimates that 250,000 people in the United States will die from cancer in 1974 and it has been speculated that 100,000 of them will die because of failure to control the primary tumour site[1]. In the report of a 1972 conference on particle accelerators in radiation therapy Bagshaw said: ". . . perhaps the most critical factor in determining success or failure in the sterilisation of a localised neoplasm with standard megavoltage radiation is the state of oxygenation of the cells being irradiated . . ."[2]. Cells which are poorly oxygenated when irradiated are more resistant to conventional X radiation than those which are well oxygenated. Many tumours are hypoxic and thus may be more resistant to radiation than the well oxygenated surrounding normal tissue. Considerable time and effort have been expended during the past decade in attempts to circumvent this problem. Efforts are currently focused on the use of heavy particles. We now have data suggesting that hyperthermia in conjunction with standard megavoltage radiation may present an alternative for circumventing the oxygen problem.

Our interest in the oxygen problem arose from *in vivo* studies of the effects of hyperthermia on the radiation sensitivity of normal and malignant tissues. These studies showed that the radiation sensitivity of C3H mammary tumours in mice was increased by a factor of 4.27 by treatment at 43.0° C, whereas the sensitivity of normal mouse skin was increased by a much smaller factor—2.06 (ref. 3). Since this tumour is considered to be poorly oxygenated, the data suggested a differential effect of hyperthermal treatment on the radiation sensitivity of hypoxic and well oxygenated cells. We present here data that show a selective hyperthermal radiation sensitisation of anoxic cells.

Our biological test system was the spleen-colony bone marrow system of Till and McCulloch[4]. Receptor mice were given a whole body dose of 800 rad which eliminated endogeneous haematopoetic cells from the spleen. On the next day, marrow cells were collected from donor mice, counted, subjected to the experimental treatment and then injected into the tail vein of receptor mice. A fraction of these cells lodged in the spleen and grew to form cell aggregates, grossly visible as nodules in the otherwise radiation-depleted spleen. The number of colonies in the spleen 10 d after injection was used as a relative measure of cell survival.

Properly diluted suspensions of bone marrow cells were placed in siliconised test tubes in water baths for 1 h at 37.5°, 42.5° or 43.0° C. This hyperthermal treatment was initiated before irradiation, maintained during irradiation and continued after irradiation, with the heating time equally divided between before and after. This treatment regimen was chosen to allow direct comparison with parallel studies of tumour therapy already in progress. Cells were irradiated with approximately 100 rad min^{-1} from a conventional 100 KVP X-ray unit.

Hypoxia was produced by causing high purity nitrogen containing 5% carbon dioxide (specified by the manufacturer as having oxygen contamination of less than 2 p.p.m.) to flow over the cell suspensions, which were agitated periodically to assure gas equilibrium. The effluent gas was monitored continuously and the degree of hypoxia was considered adequate when less than 10 p.p.m. of oxygen was detected, although the gas flow was continued for the full hour of thermal treatment. Gas treatment was identical for the oxygenated group, except that air with 5% carbon dioxide replaced the nitrogen–CO_2 mixture. Cells rendered anoxic in this manner and well-oxygenated controls were irradiated, using an appropriate dose range, to determine radiation survival curves at each of the hyperthermal temperatures. Data were obtained for both the anoxic and oxygenated groups during the same period of treatment and at least two independent treatments were carried out at each temperature. Regression analysis was carried out on the survival data to yield the survival curve slopes shown in Table 1.

Table 1 Calculated best fit slopes and associated standard deviations, obtained with hyperthermal treatment

Temperature (° C)	Survival curve slope (10^2 per rad)		OER
	Oxygenated	Anoxic	
37.5	0.94 ± .05	0.38 ± .02	2.47 ± .19
42.5	1.81 ± .18	1.07 ± .06	1.69 ± .19
43.0	2.66 ± .09	1.93 ± .09	1.38 ± .08

OER, oxygen enhancement ratio. This describes the relative radiation sensitivity of the oxygenated and anoxic cells, and was calculated from the ratios of the slopes of the anoxic to oxygenated cells at each temperature.

Although there was a marked increase in the radiation sensitivity of both oxygenated and anoxic cells with increasing temperature of treatment, it was appreciably greater for the latter. Anoxic cells subjected to the hyperthermal treatment at 43.0° C were more sensitive to radiation by a factor of 5.07 (1.93/0.38) and oxygenated cells by a factor of 2.83 (2.66/0.94) than their controls at 37.5° C. This selective radiation sensitisation brings about a reduction in the oxygen enhancement ratio (OER) from a value of 2.47 at 37.5° C to 1.38 at 43.0° C.

To place these data in perspective, Table 2 lists some representative and recently reported OER values from studies with heavy particle beams produced by an accelerator. They range from approximately 1.5 to 2.2. Since the OER of 1.38 which we have obtained through hyperthermal treatment at 43.0° C seems to compare well with those reported from heavy particle studies, our data suggest that the use of hyperthermia as an adjunct to radiation therapy might well be a way to circumvent the oxygen problem. The production of thermal fields necessary for the treatment of patients will require considerable research and engineering. If a technology for producing suitably controlled and localised thermal fields can be evolved, hyperthermia in conjunction with readily available radiation sources (such as cobalt teletherapy units, linear accelerators or betatrons) might provide some of the same advantages as heavy particle therapy.

Table 2 Recently reported OER values obtained with heavy particle beams under conditions suitable for patient treatment

Particle	OER	References
Pi mesons	1.5 –2.2	5
Fast neutrons	1.5 –1.8	6
Helium ions	1.55–1.65	7

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Functional identification of three components which mediate tissue-type specific embryonic cell adhesion

VARIOUS hypotheses have been suggested to explain *in vitro* reconstruction of tissues from populations of dispersed embryonic cells[1-5], all asserting that it is based on selective adhesion. Although selective adhesion has been amply demonstrated[6-8], its molecular basis as well as the identity and number of components involved remains to be defined. Supernatant solutions from cell and tissue cultures have been reported to yield a component(s) apparently involved in the enhancement of cell aggregation[9-11]. In addition a component(s) in the supernatant binds at the cell surface and is specific to the cell type from which the supernatant is prepared[11]. These results suggested the existence of specific receptors at the cell surface that are complementary to the aggregation promoting molecules (APM). We have now found that the binding factor participates directly in adhesion and that another component must be present for adhesion to be complete.

Collection of ^{3}H-glucosamine-labelled aggregation-promoting supernatant solutions (APMs; retina aggregation promoting material (RAPM) and cerebral lobe aggregation promoting material (CLAPM, called BAPM in ref. 11)), preparation of single cells and binding of APMs to the cell surface was achieved as before[11]. After binding (20×10^6 cells per ml of RAPM and 5×10^6 cells per ml of CLAPM), cells with APM or control cells were pelleted and resuspended in 1% glutaraldehyde in phosphate-buffered saline at *p*H 7.2 for 30 min at room temperature. The fixed dead cells were washed in the cold with six changes of Tyrode solution for 48 h before use.

After glutaraldehyde fixation, neither control cells nor those fixed with APM adhered to one another, even when brought into contact on a gyratory shaker. Cells fixed after binding of ^{3}H-APM retained approximately 50% of bound radioactivity and lost no more label during either washing or a subsequent 24 h incubation at 37° C. Thus fixation bound the APM permanently to the cells and caused no generalised stickiness which could complicate later experiments.

When added to freshly dispersed live cells of the same type, control fixed cells (no bound APM) had no effect on aggregation while APM-fixed cells enhanced the size of the aggregates formed (Figs 1 and 2). A minimum of one APM-fixed cell for each nine freshly dispersed live cells was required for maximal enhancement of retina cell aggregation while for cerebral cells the ratio was one in five. When, using these ratios of cells, the assay was carried out in the presence of 5 μg ml^{-1} cycloheximide (a concentration that inhibits 98% of ^{14}C-leucine incorporation within 10 min and 100% of aggregation of embryonic cells, our unpublished observations), fixed cells did not mediate any aggregation. This suggests that fixed cells alone do not mediate adhesion of live cells but that live cells must contribute some essential component(s), which was synthesised during our experiment. If this component were newly synthesised APM, addition of APM alone to APM-fixed cells should have caused adhesion or agglutination. This was not the case, further suggesting that the component(s) contributed by the live cells differs from the active factors in APM preparations.

Table 1 shows that fewer single cells remained in homologous combinations of live cells after 24 h with APM fixed cells than with control fixed cells. Thus APM-fixed cells interact with live cells in enhancing aggregation. We have evidence (manuscript in preparation) that APM is bound to the cell surface through the carbohydrate moiety of a macromolecule containing both carbohydrate and protein; for exhaustive pronase digestion does not affect binding of ^{3}H-glucosamine-labelled APM. To further characterise the role of the protein moiety of the surface-bound APM, APM preparation was digested with pronase before binding and fixation. As with whole APM, bound radioactivity was not removed during washing or culture. After washing, fixed cells were combined with live cells at ratios of 1:1 to 1:10. No live cells were promoted to aggregate (Fig. 1*d*). Results were similar when 10-d embryonic chick neural retina (Fig. 1) and 10-d cerebral lobe cells (Fig. 2) were combined in the appropriate combinations with fixed cells.

Taken together these results suggest that the live cells contribute a component(s) to the adhesive mechanism which differs from APM, and that its activity is mediated through the protein portion of the APM molecule. We have obtained further support

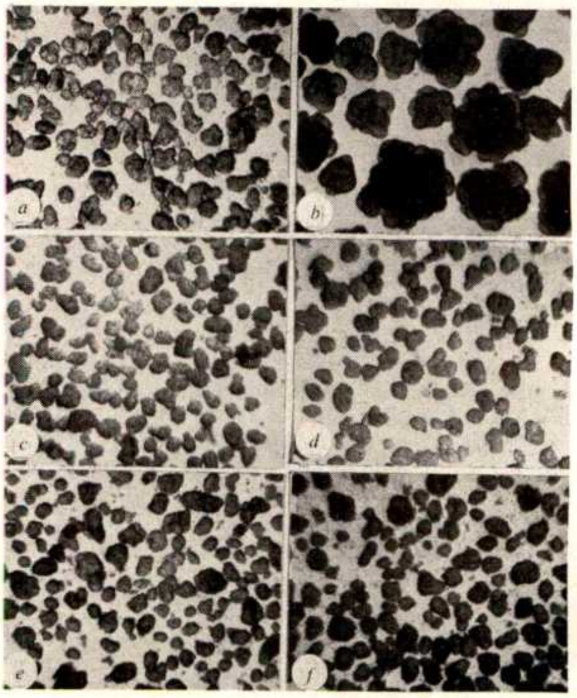

Fig. 1 Aggregation of 18×10^6 10-d retina cells in 3 ml of Eagle's basal medium under an atmosphere of 10% CO_2 in air on a gyratory shaker for 24 h. There were 2×10^6 fixed cells in in each case. *a*, No fixed cells; *b*, RAPM-fixed retina cells; *c*, control fixed retina cells; *d*, pronase-digested-RAPM-fixed retina cells; *e*, CLAPM-fixed cerebral cells; *f*, control fixed cerebral cells. (× 15).

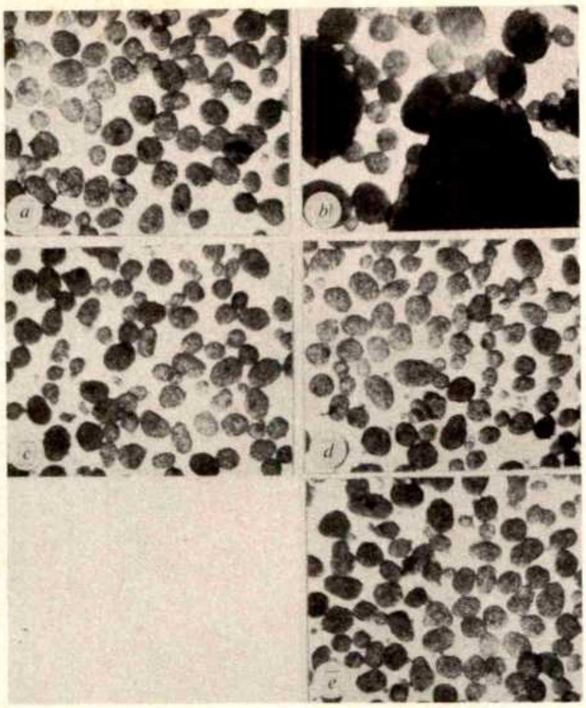

Fig. 2 Aggregation of 4 × 10^6 cerebral cells as in Fig. 1. *b–e*, 1 × 10^6 fixed cells; *a*, no fixed cells; *b*, CLAPM-fixed cerebral cells; *c*, control fixed cerebral cells; *d*, RAPM-fixed retina cells; *e*, control fixed retina cells. (× 15).

for the existence of this third component (we consider APM and its receptor to be separate components). If cells are synthesising a third type of molecule which mediates aggregation, monolayer cultures should synthesise and release it into the medium. APM-fixed cells in rotation culture on top of monolayers should thus be agglutinated in the medium above the monolayer. (Agglutinated here distinguishes loose clusters of dead cells from the formation of tissue-like aggregates of live cells. Fixed dead cells, although their adhesive mechanism may be reconstituted, would be unable to undergo further histotypic organisation.)

Monolayer cultures were prepared in Eagle's basal medium without serum; after incubation for 24 h at 37° C they were washed twice with warm serum-free culture medium, and control fixed (no APM) or APM-fixed cells were added. Incubation continued at 37° C on a gyratory shaker for 24 h (Fig. 3). APM-fixed cells were agglutinated maximally (Fig. 3*b* and *f*) while controls showed little or no agglutination (Fig. 3*c* and *g*). These results are not due to monolayer cells which were loosened from the substrate as few if any were released (Fig. 3*a* and *e*). Thus our third component can be active in a form unattached to live cells.

To test the specificity of the component(s) we prepared heterologous combinations between APM-fixed cells and live cells. Aggregation assays revealed little or no ability of APM-fixed cells to enhance aggregation even at high ratios of fixed to live cells (Fig. 1*e* and *f*, Fig. 2*d* and *e*). Rotation culture of APM-fixed cells on top of heterologous monolayers gave essentially the same results (Fig. 3*d* and *h*). Agglutination was always greatest with homologous combinations but some occurred with heterologous combinations. Counts of single cells remaining after 24 h also reflect the specificity of the proposed third component (Table 1). This is evident since in heterologous combinations the number of single cells remaining is the same for APM-fixed and control cultures, while in homologous combinations more single cells remain in control cultures than in APM-fixed cultures.

Table 1 Effect of APM-fixed cells on the number of single cells remaining in suspension after 24 h of rotation culture

Live cells	Fixed cells	Single cells remaining after 24 h	Control fixed-APM-fixed
Retina cells 18 × 10^6	RAPM-retina 2 × 10^6	2.2 × 10^6 ± 0.3 × 10^6	1.2 × 10^6
	Control retina 2 × 10^6	3.4 × 10^6 ± 0.3 × 10^6	
	CLAPM-cerebral 2 × 10^6	1.4 × 10^6 ± 0.2 × 10^6	−0.1 × 10^6
	Control cerebral 2 × 10^6	1.3 × 10^6 ± 0.3 × 10^6	
Cerebral cells 4 × 10^6	CLAPM-cerebral 1 × 10^6	0.9 × 10^6 ± 0.1 × 10^6	0.5 × 10^6
	Control cerebral 1 × 10^6	1.4 × 10^6 ± 0.1 × 10^6	
	RAMP-retina 2 × 10^6	2.8 × 10^6 ± 0.1 × 10^6	0
	Control retina 2 × 10^6	2.8 × 10^6 ± 0.3 × 10^6	

Single cells were counted on a Coulter model B particle size analyser at the appropriate settings for each cell type. These results are each the average of six experiments.

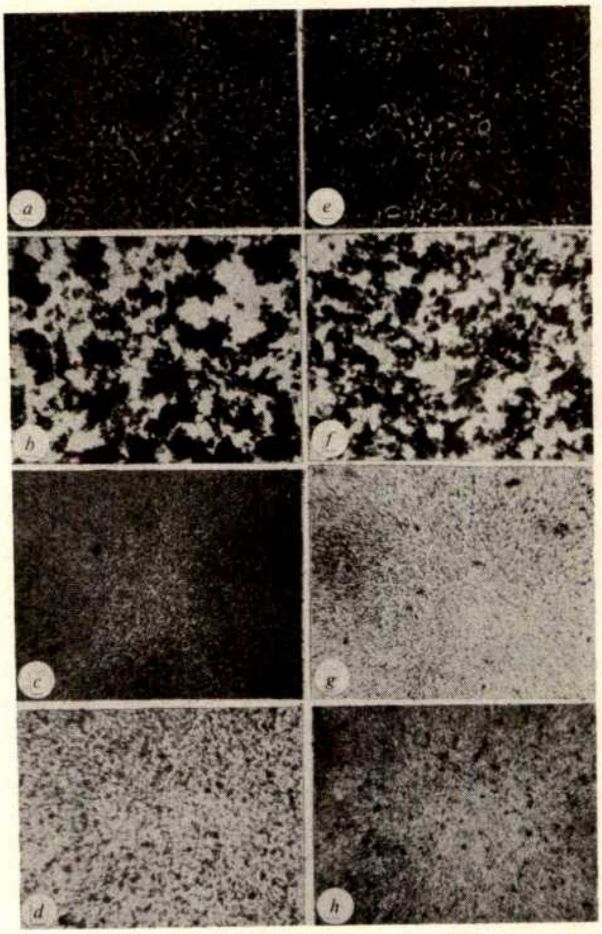

Fig. 3 Agglutination of fixed cells (2 × 10^6 retina cells or 1 × 10^6 cerebral cells) in rotation culture on monolayer cultures of retina cells (*a–d*) or cerebral cells (*e–h*) for 24 h. *a,e*, No fixed cells; *b,f*, homologous APM-fixed cells; *c,g*, homologous control fixed cells; *d,h*, heterologous APM-fixed cells. (*a,e*, × 96, focused at plane of monolayer; *b–d* and *f–h*, × 15, focused above monolayer.)

Various models are consistent with our results. For example, the third component may modify APM in some way to make it capable of forming a functional adhesion, or APM may modify the cell surface, thus allowing third component alone to form a functional adhesion. Or the third component may act as a ligator (Fig. 4). According to this model APM interacts specifically through its carbohydrate moiety with a cell surface receptor. The third component(s) interacts with the protein moiety of APM, again specifically, to effect a bridge between the cells. In this model the third component appears to be functionally

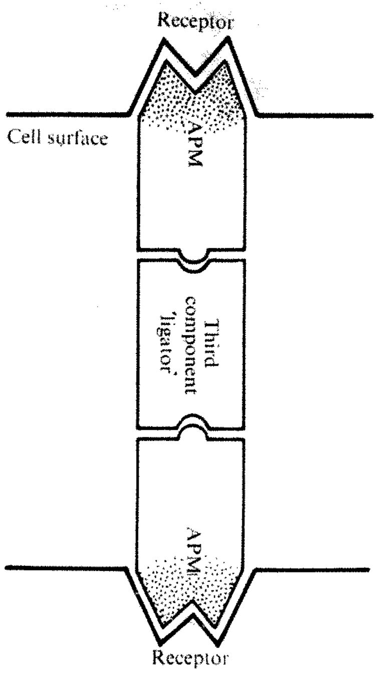

Fig. 4 Model showing the role of each of the proposed components in forming a complete specific adhesive bond. The stippled area is carbohydrate.

analogous to the sponge aggregation factor[12,13]. The aggregation-promoting factor, or APM as we term it, seems to be functionally analogous to the base plate molecule[14], in that APM and baseplate mediate the effect of the ligator molecule. Although no component analogous to our specific receptor has been identified in the sponge system, Weinbaum and Burger[14] found that once baseplate is removed from cells by hypotonic shock it can be added back to the cells, effecting a functional union.

The existence of the third component(s) may explain the necessity for biosynthetic activity even when APM is supplied to the cells[9]. Immediately after trypsinisation both APM and the third component are removed from the cell surface, for control fixed cells can neither enhance the aggregation of live cells nor be agglutinated when placed on a monolayer of live cells. Aggregation thus requires the replacement of both APM and third component. When APM is supplied a complete adhesive bond is still not formed; the third component is now the limiting factor. Our preliminary observations suggest that this third component is extremely labile. This observation may bear on the need for continued biosynthetic activity during aggregation. If this component is continuously destroyed during aggregation then its continued synthesis would be necessary.

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TRH potentiates behavioural changes following increased brain 5-hydroxytryptamine accumulation in rats

WHEN tryptophan is administered to rats pretreated with a monoamine oxidase (MAO) inhibitor there results a characteristic syndrome of hyperactivity. This syndrome seems to be due to the increased synthesis and accumulation of the putative neurotransmitter 5-hydroxytryptamine (5-HT) and its 'spill-over' into functional activity which is presumably dependent on the stimulation of postsynaptic 5-HT receptors[1]. A qualitatively similar syndrome is produced by the administration of 5-methoxyN,N-dimethyltryptamine which, it has been suggested, directly stimulates 5-HT postsynaptic receptors[2]. This hyperactivity syndrome has been used to investigate the functional organisation of brain 5-hydroxytryptamine, the action of drugs such as chlorpromazine[2], reserpine[1] and lithium[3] and to investigate the role of brain catecholamines[4] in the mediation of brain 5-HT function.

Recently a report[5] has suggested that thyrotrophin releasing hormone (TRH) produced a rapid, though transient, improvement in depression when given intravenously. Because the combination of tryptophan plus a monoamine oxidase inhibitor has antidepressant properties[6] and because reserpine[7] and lithium[8,9] also affect mood in man, we investigated the effect of TRH on the hyperactivity syndrome.

Rats were pretreated intraperitoneally with 2 mg kg^{-1} TRH (Calbiochem, London) 3 h before the administration of 20 mg kg^{-1} tranylcypromine (Tcp), 30 min after which L-tryptophan was given. As shown in Fig. 1*a*, TRH caused an increase in the degree of hyperactivity[1]. There was no potentiation with a TRH dose of 0.25 mg kg^{-1}, perceptible potentiation with 0.5 mg kg^{-1}, a maximum potentiation at 1 mg kg^{-1} and 2 mg kg^{-1} but no potentiation when the dose was increased to 10 mg kg^{-1}. With a dose of 1 mg kg^{-1} it was observed that there was some potentiation of hyperactivity when TRH was given 1 h before Tcp and tryptophan, a marked response after 3 h and 6 h, but no effect at 24 h. A 1 mg kg^{-1} dose of an equimolar solution of proline, histidine and glutamic acid injected instead of TRH (pyroglutamyl histidyl prolylamide) failed to produce potentiation, showing that the constituent amino acids in combination were ineffective. No hyperactivity occurred after TRH pretreatment when either Tcp or tryptophan alone were administered.

Pretreatment with either thyroid stimulating hormine (TSH, 1 mg kg^{-1}) or L-thyroxine (1 mg kg^{-1}) (both from Sigma, London) 3 h before the Tcp, with L-tryptophan after a further 30 min failed to produce potentiation of the hyperactivity suggesting that TRH was not producing its effect by its action on pituitary secretion of TSH by a TSH-mediated effect on thyroid function. This was confirmed by the observation that TRH still potentiated the hyperactivity in rats hypophysectomised 48 h previously.

TRH alone had no effect on brain tryptophan or 5-HT concentrations nor did it affect brain tryptophan concentrations, 5-HT concentrations or the rate of brain 5-HT accumulation after the administration of Tcp and L-tryptophan. The effect of TRH on the hyperactivity produced by 5-methoxy N,N-dimethyltryptamine (5-MeODMT) was therefore examined to see whether TRH was effecting the postsynaptic response to activation of 5-HT receptor sites.

Rats were pretreated with TRH (1 mg kg^{-1}) followed 3 h later by Tcp (20 mg kg^{-1}) with 5-MeODMT (2 mg kg^{-1}) after a further 30 min TRH pretreatment consistently increased the degree of hyperactivity which was prolonged following 5-MeODMT (Fig 1*b*) This suggested that TRH was altering either 5-HT receptor sensitivity or the postsynaptic responses to increased 5-HT release When rats were pretreated with TRH (10 mg kg^{-1}) and subsequently challenged with Tcp and 5-MeODMT as above no potentiation was seen This is consistent with the observation that this higher dose of TRH does not potentiate the hyperactivity following Tcp and L-tryptophan

Previous experiments have shown the importance of brain dopamine in the production of the hyperactivity following increased 5HT synthesis or 5-MeODMT The results were tentatively interpreted as indicating that there may be a group of dopaminergic neurones lying between the 5-HT neurones initiating the response and those mechanisms responsible for the expression of the syndrome[4] Furthermore, Plotnikoff *et al*[10] showed that TRH potentiates the hyperactivity resulting from administration of a MAO inhibitor and L-dopa, a syndrome which is nevertheless qualitatively different from that produced by increasing 5-HT synthesis It was possible therefore that TRH might affect the 5-HT induced hyperactivity by an action on the postsynaptic dopaminergic system postulated We therefore attempted to confirm the visual scoring of Plotnikoff *et al*[10] by activity meter measurements

Pretreatment with TRH 3 h before the Tcp and L-dopa potentiated the hyperactivity, both at a TRH dose of 1 mg kg^{-1} and 0 25 mg kg^{-1} (Fig 2) This lower dose had been ineffective in enhancing Tcp/L-tryptophan hyperactivity This dose, however, did potentiate hyperactivity following Tcp and DL-5-hydroxytryptophan (50 mg kg^{-1}) We are unable, at present, to suggest reasons for these dose-response differences

There was no alteration in whole brain concentrations of dopamine and noradrenaline 3 h after TRH administration or after a further 90 min when Tcp and L-tryptophan had also been administered

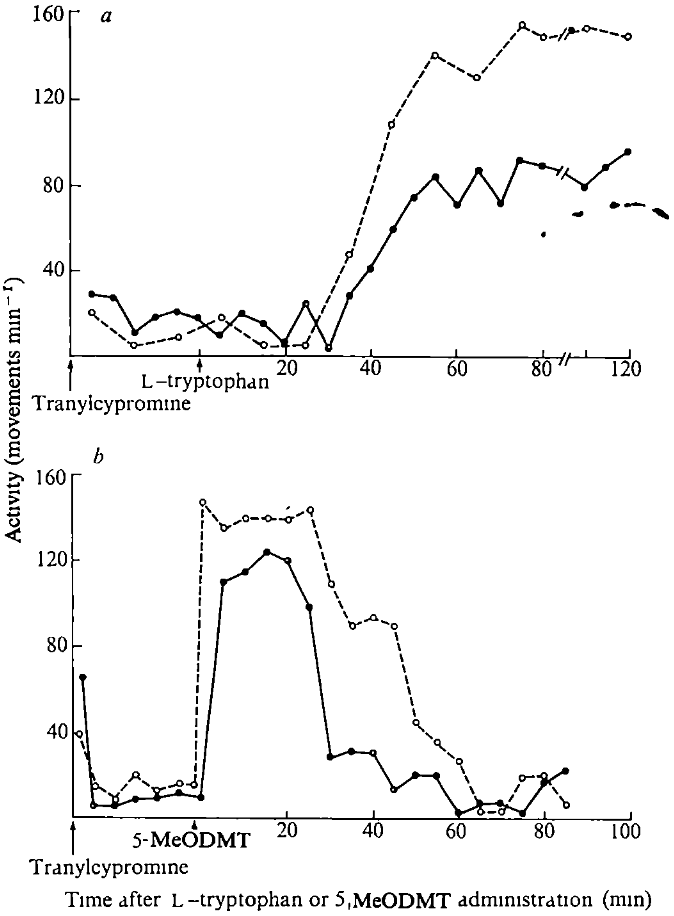

Fig 2 Hyperactivity response to L-dopa following TRH Rats pretreated with 1 mg kg^{-1} TRH (○), 0 25 mg kg^{-1} (△) or saline (●) 3 h later all groups were injected with tranylcypromine (20 mg kg^{-1}) with L-dopa (50 mg kg^{-1}) administered 30 min later

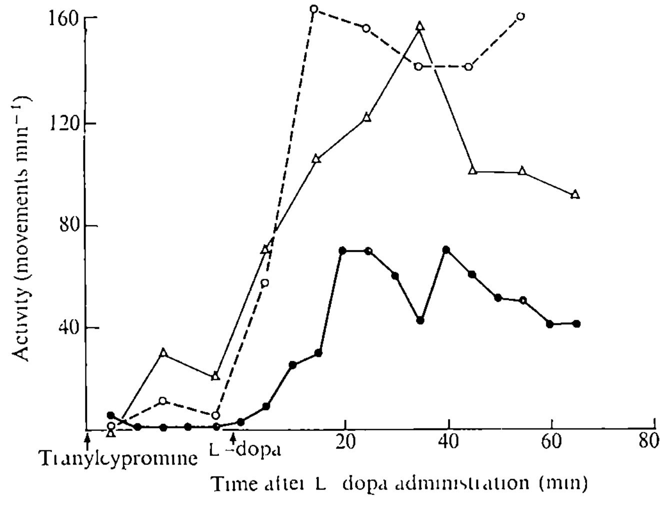

Fig 1 Hyperactivity response to, *a*, L-tryptophan and, *b*, 5-methoxyN,N-dimethyltryptamine (5-MeODMT) following TRH Male Wistar rats (150–200 g) were used in all experiments and were pretreated with either TRH 2 mg kg^{-1} (○) or saline (●) 3 h later both groups were given tranylcypromine (20 mg kg^{-1}) with L-tryptophan (50 mg kg^{-1}) or 5-MeODMT (2 mg kg^{-1}) 30 min later Activity was measured in groups of three rats on Animex activity meters (sensitivity and tuning setting 30 μA) with total movements recorded each minute Results are plotted against time after tranylcypromine administration and show typical results of experiments performed at least three times

There have now been several reports of alterations in behavioural responses to released central neurotransmitters following administration of hypothalamic hormones Plotnikoff *et al*[10] reported that TRH enhanced the hyperactivity following MAO inhibition and L-dopa and the same group[12] found similar enhancement when rats were pretreated with melanocyte stimulating hormone inhibitory factor (MIF) another hypothalamic hormone with suggested antidepressant properties[13] MIF also elicits stereotyped behaviour in cats indistinguishable from that following L-dopa or amphetamine[14] Since amphetamine effects dopaminergic systems in the brain[15], MIF may thus be altering the function of dopaminergic neurones This may also be the case with TRH since we have found that the hyperactivity resulting from injection of 4-methoxyamphetamine (10 mg kg^{-1}) 30 min after Tcp (20 mg kg^{-1}) was also potentiated by pretreatment 3 h previously with TRH (1 mg kg^{-1})

Another possibility examined was that TRH might increase the overall sensitivity of neurones to excitatory stimuli We therefore pretreated groups of mice with TRH (1 mg kg^{-1}) or saline and 3 h later pentylenetetrazol (Leptazol, 70 mg kg^{-1}) was given intraperitoneally In the animals which had received TRH, convulsions occurred with a shorter latent period than those receiving saline (Fig 3) At present, however, it is not possible to exclude the possibility that this effect of TRH is mediated by some change in the function of brain monoamines

Recently it has been reported[16] that TRH increased the turnover of cerebral noradrenaline No change in the brain content of 5-HT, noradrenaline, dopamine, homovanillic acid or 5-hydroxyindoleacetic acid was found in this study The TRH dose used, however (10 mg kg^{-1}) caused no potentiation of the hyperactivity following Tcp and either L-tryptophan or

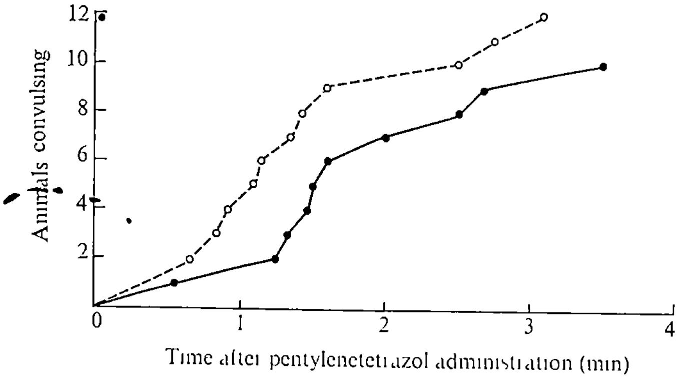

Fig 3 Time taken for a group of 24 mice given either 1 mg kg^{-1} TRH (○) or saline (●) to convulse following injection of pentylenetetrazol (70 mg kg^{-1}) 3 h later All 12 animals injected with TRH convulsed whereas only 10 of the 12 saline injected animals convulsed Therefore nonparametric statistics (Mann-Whitney test[11]) were used for analysis TRH-treated mice convulsed with a significantly shorter latent period than control mice ($T<0$ 01)

5 MeODMT and we have so far been unable to definitely incriminate noradrenaline in the hyperactivity response to increased 5-HT accumulation[4]

These results show that TRH, in a curiously restricted dose range, causes potentiation of the hyperactivity response to stimulation of 5-HT receptors in the brain Whether this is due to some effect of TRH to alter neuronal sensitivity generally, as might be inferred from the potentiation of the various effects of L-dopa and Tcp, 4-methoxyamphetamine and pentylenetetrazol—or more specifically through an effect on a system common to all these stimuli—cannot at present be concluded

These experiments and those of others show conclusively, however, that TRH has actions on brain function quite independent from its effects on TSH and therefore thyroid function

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γ-Aminobutyric acid synthesised in the olfactory nerve

WE have found the candidate neurotransmitter γ-aminobutyric acid (GABA) and its biosynthetic enzyme in the olfactory nerve of two fish In the light of these findings current concepts of GABA as an exclusively inhibitory transmitter may have to be reconsidered The olfactory nerve is a component of the rhinencephalon, a phylogenetically primitive part of the central nervous system (CNS) In addition to its receptive sensory function, the olfactory nerve conducts impulses Fish have either short olfactory nerves and long olfactory tracts, as do most vertebrates, or long nerves and short tracts, as found in the gar fish (*Lepisosteus osseus*) and the pike (*Esox estor*)[1] The olfactory cilia and nerve cell body are in the olfactory mucosa, from where the nerve action potentials are propagated to the olfactory bulb, 3 cm (pike) to 15 cm (gar) away, depending on the size of the animal The primary synapse occurs within the olfactory bulb with the mitral cells The olfactory nerve in the gar and the pike contains no motor nor other known sensory fibres[2,3] Furthermore, it is neurally homogeneous, sensory, unmyelinated and is of a reasonable size for biochemical study (50–150 mg per nerve per fish)

Fish were caught by net in the Mississippi River, stored and transported to the laboratory in small pools and used as needed The general methodology of Hildebrand *et al*[4] was used to test for the biosynthesis of acetylcholine, dopamine, noradrenaline, adrenaline, serotonin and γ-aminobutyric acid Gar nerve explants or brain slices (20 mg) were incubated in 500 µl gar Ringer (112 mM NaCl, 3 5 mM KCl, 3 mM $CaCl_2$, 24 mM glucose and 4 mM N Tris (hydroxymethyl)methyl-2-amino-ethane sulphonic acid) containing 5 µCi of either ^{14}C-L-tyrosine, ^{14}C-L-tryptophan, ^{14}C-choline or ^{14}C-L-glutamate (each 50 Ci mol^{-1}) After 3 h at ambient temperature (23° C), tissues were blotted, homogenised (glass-to-glass) in 120 µl 0 5 N formic acid containing carrier and subjected to paper electrophoresis to resolve the possible neurotransmitter from the labelled precursor There was no detectable incorporation of tyrosine into dopamine, noradrenaline or adrenaline, of tryptophan into serotonin, nor of choline into acetylcholine in the olfactory nerve On the other hand, there was substantial incorporation of L-glutamate into a substance which comigrated with GABA on paper electrophoresis Labelled dopamine, noradrenaline, serotonin, acetylcholine and GABA were formed in the brain slices from their precursors The demonstration of GABA formation in gar olfactory nerve prompted an investigation of its metabolism *in vitro*

GABA is an established inhibitory neurotransmitter acting at the crustacean neuromuscular junction and a probable neurotransmitter in the vertebrate CNS[5,6] The biosynthesis of GABA from L-glutamate is catalysed by glutamate decarboxylase (EC 4 1 1 15) and the concentration of GABA parallels that of its biosynthetic enzyme[5] We found glutamate decarboxylase activity in homogenates of olfactory nerve (Table 1) at 25% of the level found in the brain, although the specific activity in the pike (per unit weight of tissue) was about half that in the gar Enzyme activity in other tissues was very low or not detected (<5 nmol min^{-1} g^{-1}) Glutamate decarboxylase activity was also present in the gut homogenate from the pike, but not the gar Roberts *et al* have reported that a second glutamate decarboxylase (GAD II) is present in non-nervous tissue[8,9] The activity might also represent enzyme present in bacterial flora although we removed the flora from the tissue by extensive rinsing before homogenisation

When we measured GABA as described before[10] (Table 2) we found that nerves contained 65% and 75% of the amount found in the brain of the pike and gar, respectively None was found in several other tissues (Table 2) except for the gut of the pike The GABA content thus parallels that of glutamate decarboxylase (Table 1) The level of GABA in the fish brains was about 50–60% that of rat brain[11] It was similar to that found in the mammalian CNS[12] but not that in the inhibitory

Table 1 Glutamate decarboxylase activity

Tissue	Gar (nmol min^{-1} g^{-1})	Pike (nmol min^{-1} g^{-1})
Olfactory nerve	80 1	40 9
Brain (whole)	320	162
Gill	<5	<5
Visceral fat	<5	<5
Gut	<5	17 4
Skeletal muscle	<5	NS

A 1 10 tissue homogenate was made in 5 mM potassium phosphate (*p*H 7 4)-0 1 mM EDTA (buffer *A*) Then 10 µl of extract was added to 10 µl of solution so that the final incubation mixture contained 2 0 mM ^{14}C-L-glutamate (20 Ci mol^{-1}), 0 1 mM pyridoxal phosphate and 50 mM potassium phosphate (*p*H 7 4) After incubation for 5 min at 37° C, 10 µl of 0 5 N formic acid-25 mM GABA was added to stop the reaction Then 10 µl aliquots were taken for paper electrophoresis[7] After drying at 100° C for 15 min, the GABA zones were developed with ninhydrin spray and cut out for radioactivity measurement by liquid scintillation counting[7]

NS, not studied

motor neurones of the peripheral nervous system of the lobster (about 100 µmol g^{-1}) (ref 13)

Two mitochondrial enzymes are required for GABA catabolism The first, GABA-α-oxoglutarate transaminase, and the second, succinate semialdehyde dehydrogenase, catalyse reactions 1 and 2, respectively.

$$\text{(1)}\quad \begin{matrix}\text{GABA} & & \text{succinate semialdehyde}\\ + & \leftrightharpoons & +\\ \alpha\text{-oxoglutarate} & & \text{L-glutamate}\end{matrix}$$

$$\text{(2)}\quad \begin{matrix}\text{succinate semialdehyde} & & \text{succinate}\\ + & \leftrightharpoons & +\\ \text{NAD} & & \text{NADH}\end{matrix}$$

These enzymes are ubiquitous in tissue, although they are most active in nervous tissue[14] The activity of GABA transaminase was highest in brain and olfactory nerve, but present in all tissues assayed (Table 2)

Glutamate decarboxylase is often considered a marker for neurones for which GABA is the neurotransmitter[5], and so we characterised it in the olfactory nerve In gar and pike olfactory nerve homogenates, the time course of GABA biosynthesis was linear for more than 20 min at 37° C (see legend to Table 1) In each case, activity was proportional to protein concentration up to 5 5 mg protein per ml of incubation medium (higher protein concentrations were not tested)

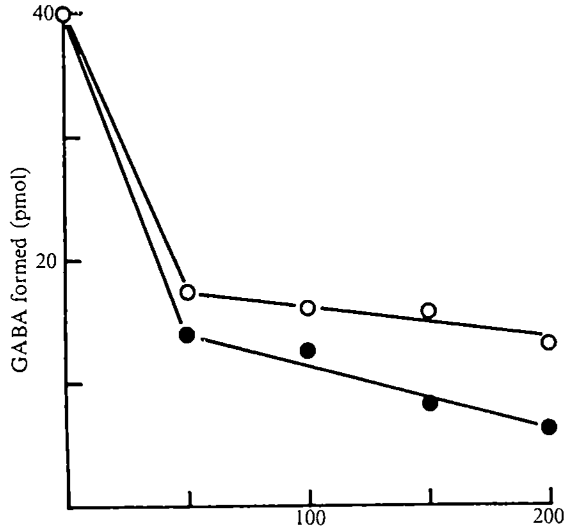

Fig 1 Inhibition of pike olfactory nerve glutamate decarboxylase by salt Assays were carried out as described in Table 3 using the specified concentration of KCl (●) or NaCl (○)

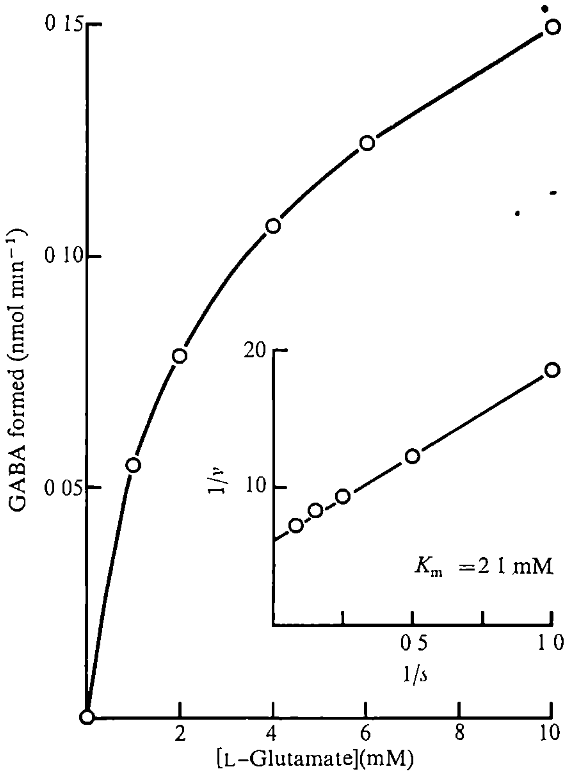

Fig 2 GABA biosynthesis in pike olfactory nerve as a function of glutamate concentration Assays were carried out as described in Table 4 using the specified concentration of glutamate

The mammalian and lobster glutamate decarboxylase enzymes, which require pyridoxal phosphate as a cofactor, are inhibited by carbonyl-trapping reagents[15] Table 3 shows that glutamate decarboxylase activity in the olfactory nerve extract of the pike is inhibited markedly by hydroxylamine Semicarbazide, thiosemicarbazide and isoniazid are also inhibitory Results were similar with the gar olfactory nerve (not shown) These studies support the hypothesis that the glutamate decarboxylase of the olfactory nerve requires a carbonyl-containing group for activity After dialysis overnight against 2,000 volumes of buffer, pyridoxal phosphate stimulated GABA biosynthesis by 20% As in the case of the mammalian and lobster enzymes[15], the data suggest that the pyridoxal phosphate is rather tightly bound to the enzyme

Mouse brain glutamate decarboxylase is inhibited by chloride[16], while the mouse kidney enzyme[9] (GAD II) and the lobster inhibitory nerve enzyme are not[17] The data from Fig 1

Table 2 Levels of GABA and its transaminase in tissues of gar and pike

Tissue	GABA Gar (nmol g^{-1})	GABA Pike (nmol g^{-1})	GABA transaminase Gar (nmol min^{-1} g^{-1})	GABA transaminase Pike (nmol min^{-1} g^{-1})
Olfactory nerve	0 55	0 49	46 3	24 1
Brain	0 74	0 75	87 9	50 3
Gill	<0 02	<0 02	34 4	7 6
Visceral fat	<0 02	0 03	11 0	9 8
Gut	<0 02	0 26	25 1	11 6
Skeletal muscle	NS	NS	22 1	NS

To assay GABA transaminase, tissues were homogenised in buffer *A* To remove low molecular weight endogenous substrates, the extracts were dialysed against 100 volumes of buffer *A* for 1 h, against buffer *A* containing 100 mM KCl for 4 h and 12 h and against buffer *A* again for 1 h (all at 4° C) before assay The GABA dependent conversion of labelled α-oxoglutarate into glutamate was measured[14] and the labelled glutamate was resolved from precursor by paper electrophoresis

NS, not studied

Table 3 Inhibition of pike olfactory nerve glutamate decarboxylase by carbonyl-trapping reagents

Addition	Activity (pmol ^{14}C-GABA formed)
Control	35.9
Hydroxylamine, 0.2 mM	0.2
Isoniazid, 50 mM	4.8
Thiosemicarbazide, 12.5 mM	10.8
Semicarbazide, 50 mM	1.5

The assays were carried out as described in Table 1 using 10 µl extract (11 mg protein ml^{-1}) for a 15 min incubation at 37° C in the presence of the specified reagent.

show that sodium and potassium chloride inhibit the decarboxylase activity of the olfactory nerve. The KCl consistently inhibited activity more than NaCl, and parallel results were obtained with the gar olfactory nerve homogenate (not shown).

The olfactory nerve decarboxylase activity exhibits saturation kinetics (Fig. 2). The apparent K_m for the pike decarboxylase is 2.1 mM (Fig. 2) and that for the gar, 2.6 mM. Because crude dialysed homogenates were used, these values are not significantly different. The *p*H optimum for both the pike and gar enzymes, using 50 mM potassium phosphate buffer, is between 7.0 and 7.4.

In vertebrate neurones, the activity of the enzymes which catalyse neurotransmitter biosynthesis, and the neurotransmitter content is greatest in the synaptic region. Subcellular fractionation has shown that the biosynthetic enzyme activity and the content of acetylcholine[18-20], GABA[21] and noradrenaline[22,23] is greatest in the pinched off nerve ending, or synaptosome fraction. GABA and glutamate decarboxylase were measured in segments of the gar and pike olfactory nerves, cut progressively from the rostral to caudal ends. The enzyme activity, and the GABA content, show a rostral-to-caudal gradient with the greatest quantity nearest the synaptic region. To avoid adventitious contamination by non-olfactory nerve neurones, the synaptic region itself was not included for assay in the most caudal segment.

In the olfactory nerve of the gar and pike, GABA has several possible, and not necessarily mutually exclusive, functions. First, it might be a substrate for oxidative metabolism[15]. For example, conversion to succinate semialdehyde followed by oxidation to succinate yields 3 ATP equivalents. This GABA-shunt pathway accounts for about 10% of O_2 consumption in rat brain[15]. Second, GABA and its biosynthetic enzyme might be associated with the non-neuronal Schwann cells of the olfactory nerve. GABA and glutamate decarboxylase are found in mammalian glial cells grown in tissue culture[24]. Unlike the olfactory nerve decarboxylase, however, the glutamate decarboxylase found in gliomas (GAD II) is not inhibited by salt or by carbonyl trapping reagents[8]. Third, GABA might be taken up by the olfactory nerve from synapses in the olfactory bulb region as part of a GABA-inactivating mechanism such as for the Schwann cell and connective tissue in the lobster neuromuscular junction.

Although very unlikely, based on current concepts of its function, GABA might be a sensory excitatory neurotransmitter. It has been implicated so far exclusively as an inhibitory transmitter agent. Iontophoresis of GABA on to cerebellar and cerebral neurones[26], neurones in the dorsal Dieter's nucleus[17] and in the lobster neuromuscular junction[28], all produce inhibition. Furthermore, experiments with the olfactory bulb of the cat and rabbit are consistent with the notion that granular cell inhibition of the mitral cell is mediated by GABA[29,30]. On the other hand, the olfactory nerve in these preparations excites the mitral cells.

The presence of GABA, and its biosynthetic enzyme, in an axonal-to-synaptic gradient in the olfactory nerve of two fishes (Table 4) makes it a neurotransmitter candidate at the synapse between the nerve and the post-junctional cell in these species. Further experiments showing that GABA is released during neuronal activity and that it has the same effect on the post-synaptic cells as the natural transmitter are required before this compound can be considered a neurotransmitter substance. Until such time as the function is established, GABA and glutamate decarboxylase activity cannot be used to map inhibitory pathways in the vertebrate central nervous system.

Since this report was submitted, Flock and Lam, using methodology similar to our own, reported GABA synthesis in the sensory hair cells of amphibian and fish inner ear sense organs and fish lateral line organs[31]. The proposed function of GABA in these sensory transducing hair cells parallels that considered here for the olfactory nerve. On the other hand, Margolis suggested that carnosine may be related to olfactory nerve transmission in mouse[32].

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Table 4 Axonal-to-synaptic gradient of GABA and glutamate decarboxylase activity in olfactory nerve

	GABA (µmol g^{-1})		Glutamate decarboxylase (nmol min^{-1} g^{-1})	
	Gar	Pike	Gar	Pike
Rostral	0.37		43	
		0.14		29
	0.41		67	
	0.58		93	
		0.59		77
Caudal	0.82		111	

Assays were carried out in the rostral-to-caudal quarters of the gar and halves of the pike olfactory nerves as described in Tables 1 and 2.

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Regulation of striatal acetylcholine concentration by dopamine receptors

IMPAIRMENT of the coordinated activities of cholinergic and dopaminergic neurones in the brain has been implicated in the pathophysiology of Huntington's chorea, drug-induced dyskinesias, Parkinsonism, schizophrenia and manic-depressive psychosis[1-6] Physostigmine, a drug which increases brain acetylcholine by inhibiting acetylcholinesterase, has been reported to improve the neurological status of patients with Huntington's chorea, tardive dyskinesias and L-dopa-induced dyskinesia[7-9] The hyperkinetic involuntary movements in these disorders are thought to be related to a denervation hypersensitivity of dopamine receptors in the striatum[10] On the other hand, in diseases with decreased dopaminergic activity, such as idiopathic and phenothiazine-induced Parkinsonism, intravenous injection of small doses of physostigmine exacerbated rigidity and tremors[11,12] A similar aggravation of Parkinsonian-like symptoms by physostigmine can be observed in rats pretreated with phenothiazines or reserpine and in dogs pretreated with intracisternal 6-hydroxydopamine[13 14] L-Dopa blocks the adverse neurological effects of physostigmine in Parkinsonian patients and in animals pretreated with reserpine or 6-hydroxydopamine[11-14] These observations suggest that the response to central cholinergic stimulation depends on the functional activity of inhibitory dopamine receptors in the striatum The diminished striatal dopamine receptor stimulation in Parkinsonism[15] may secondarily produce cholinergic hyperactivity in the striatum, the converse may exist in dyskinetic and choreiform disorders Striatal dopamine receptors could regulate cholinergic activity by modulating intrastriatal acetylcholine synthesis and release by cholinergic neurones We have now tested this hypothesis by investigating the effect of dopamine receptor agonists and antagonists on striatal acetylcholine concentration

Male Sprague Dawley rats (180–200 g) were kept under constant diurnal lighting and temperature conditions for at least one week before use Animals were killed at approximately the same time (0900–1200h) of day All drugs were administered intraperitoneally Control rats received an equal volume of vehicle Rats were decapitated at various times (Table 1) after the injection of apomorphine (10 mg kg^{-1}), trivastal (10 mg kg^{-1}), *d*-amphetamine (10 mg kg^{-1}), chlorpromazine (15 mg kg^{-1}), haloperidol (10 mg kg^{-1}) and pimozide (10 mg kg^{-1}) L-Dopa (100, 300, 600 mg kg^{-1}) was administered 1 h after MK 486 (α-methyl dopa hydrazide, 25 mg kg^{-1}) and the rats were killed 1 h after the second injection

The brains were removed from the skull and placed immediately in cold pentane (−5° C) The striatum was dissected by the method of Glowinski and Iverson[16] Bilateral striata from two rats were homogenised together in 0 4 N perchloric acid (4° C) containing propionylcholine iodide as an internal standard Acetylcholine was extracted and assayed by gas-chromatography[17,18], modified as described previously[19]

The concentration of acetylcholine in the striatum had increased significantly 0 25, 0 5 and 1 h after the administration of apomorphine, trivastal and *d*-amphetamine (all $P < 0\ 001$, Table 1) The maximum increase with apomorphine and trivastal was 49% and 83% respectively after 0 5 h and for *d*-amphetamine 33 6% after 1 h Chlorpromazine, haloperidol and pimozide significantly (all $P < 0\ 001$) decreased striatal acetylcholine concentration 0 25, 0 5 and 1 h after injection The maximum reduction with chlorpromazine and pimozide was 43% and 51 2%, respectively, after 0 25 h and with haloperidol 57 9% after 30 min A significant reduction in acetylcholine concentration persisted for 4 h after injection of pimozide and for 16 h after injection of haloperidol (Table 1) L-Dopa (100, 300 and 600 mg kg^{-1}) significantly ($P < 0\ 001$) increased acetylcholine concentration in the striatum of rats pretreated with the peripheral dopa decarboxylase inhibitor MK 486 (Fig 1) MK 486 or L-dopa, when administered alone had no significant effect on striatal acetylcholine concentration

Dopamine, released from nigro-striatal nerve endings, reduced striatal neuronal discharge frequency[20 21] Since the striatum contains the highest concentration of acetylcholine[22] in the brain it is possible that intrastriatal cholinergic neurones which contain dopamine receptor sites are inhibited by dopamine Our results support this hypothesis Dopamine receptor agonists like apomorphine, trivastal, *d*-amphetamine and MK 486+L-dopa (which increases brain dopamine) may inhibit striatal cholinergic activity, which would reduce the release of acetylcholine from cholinergic neurones and produce the observed increase in striatal acetylcholine concentration In contrast, dopamine receptor antagonists such as chlorpromazine, haloperidol and pimozide would increase discharge frequency of striatal cholinergic neurones, increase the release of striatal acetylcholine and produce the observed decrease in striatal acetylcholine concentration The decrease in striatal acetylcholine concentration is not likely to be due to a central anticholinergic action of these neuroleptic drugs since chlorpromazine actually enhances the tremorigenic effect of physostigmine both in man and animals[12,13] Anticholinergic drugs which decrease brain acetylcholine also reduce the tremorigenic action of physostigmine[23] Furthermore, we have observed that pretreatment with L-dopa (300 mg kg^{-1}) significantly blocked the chlorpromazine-induced

Table 1 Effects of drugs on concentration of acetylcholine in the striatum

Drug	Dose (mg kg^{-1})	Time after injection						
		0 h	0 25 h	0 5 h	1 h	4 h	16 h	24 h
Apomorphine	10	29 6 ± 1 0	41 2 ± 1 1	44 1 ± 2 3	38 2 ± 2 8	25 0 ± 1 3	—	—
Trivastal	10	31 1 ± 1 6	52 2 ± 0 8	57 0 ± 4 3	48 3 ± 5 2	29 4 ± 2 2	—	—
d-Amphetamine	10	30 9 ± 0 4	34 6 ± 1 3	41 3 ± 1 8	42 9 ± 1 2	29 6 ± 0 7	—	—
Chlorpromazine	15	30 8 ± 1 1	17 6 ± 2 6	20 0 ± 1 3	23 6 ± 1 8	28 6 ± 1 8	—	—
Haloperidol	10	30 9 ± 0 6	17 4 ± 1 5	13 0 ± 1 4	16 7 ± 0 7	18 0 ± 0 8	24 3 ± 1 0	25 8 ± 1 2
Pimozide	10	29 3 ± 1 1	14 3 ± 1 4	14 5 ± 1 8	17 5 ± 1 3	18 5 ± 1 0	30 6 ± 0 8	—

Figures are nmol g^{-1}

Acetylcholine concentration in the rat striatum after the intraperitoneal administration of either dopamine receptor agonists or antagonists Each value is the mean ± s e of 4–15 observations

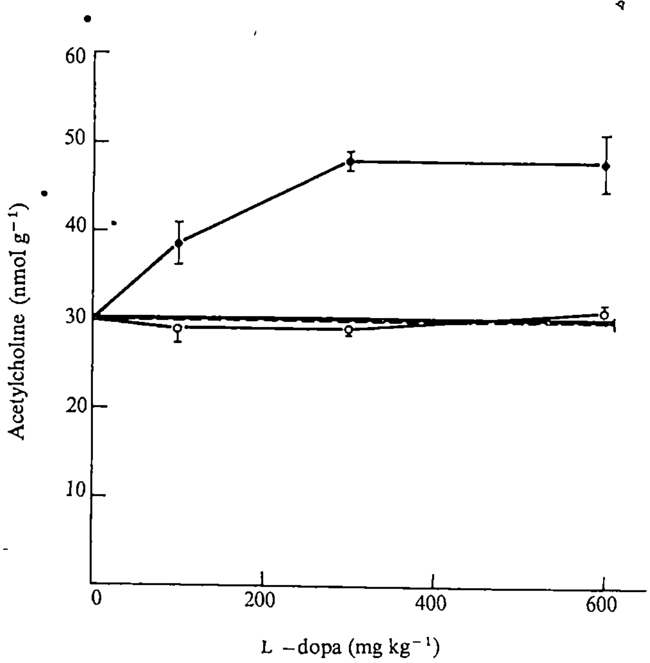

Fig. 1 Effect of various doses of L-dopa on striatal acetylcholine concentration in control and MK 486-treated rats Each point is the mean ±s e of four to twelve determinations — — —, Control, ———, MK 486, O, L-dopa, ●, MK 486 +L-dopa

decrease in striatal acetylcholine concentration, indicating that the changes in acetylcholine concentration are mediated by functional changes in the dopamine receptors

Our results are consistent with the demonstration by Stadler *et al* [24] that chlorpromazine increased acetylcholine release from the caudate nucleus of the cat, and that this release was blocked by apomorphine However, Jones *et al* [25] found no alterations in acetylcholine release during superfusions of the ventricular surface of the caudate nucleus in cats treated with either L-dopa or amphetamine Reserpine, a drug which decreases brain dopamine, also lowers striatal acetylcholine and this effect is reversed by pretreatment with L-dopa[26]

In idiopathic and phenothiazine-induced Parkinsonism there is a decrease in striatal dopaminergic activity and an apparent increase in cholinergic activity Further enhancement of cholinergic activity by the injection of acetylcholine into the basal ganglia of Parkinsonian patients was found to increase tremor on the contralateral side, while anticholinergic drugs reduced the tremor[27] Excessive discharge frequency of striatal cholinergic neurones associated with increased release of acetylcholine and decreased acetylcholine concentration may be causally related to the Parkinsonian side effects of the neuroleptic drugs This mechanism would account for the therapeutic effectiveness of anticholinergic drugs in drug-induced Parkinsonism

A stimulatory effect of neuroleptic drugs on cholinergic neurones could also be relevant to their therapeutic effectiveness in schizophrenia Several clinical studies have demonstrated that drugs which stimulate central cholinergic neurones improve schizophrenic symptomatology Cholinomimetic compounds like arecoline and physostigmine have been observed to produce symptomatic improvement in schizophrenic patients[28,29] On the other hand, anticholinergic drugs, L-dopa and methylphenidate aggravate the symptoms of schizophrenia[30-32] Physostigmine reversed the intensification of the schizophrenic psychosis produced by methylphenidate[33] The anti-schizophrenic action of phenothiazine drugs, haloperidol and pimozide could be related to their ability to block an excessive dopaminergic inhibition of cholinergic neurones in the brain

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Quantitation of adrenaline in rat brain nuclei and areas by mass fragmentography

THE existence of adrenaline (A) in the mammalian central nervous system (CNS) was first demonstrated by von Euler[1] and Holtz[2] Subsequently, many reports have documented the presence of whole brain A in mammals and amphibia In amphibia adrenaline is the major CNS catecholamine[3], being about four times greater than noradrenaline[4], while in mammals, adrenaline is only a small fraction (4–22%) of the total noradrenaline content[5 6] These data in mammals could indicate either adrenaline uptake from the periphery or a very discrete adrenaline pathway possibly with a high turnover rate We have quantitatively measured adrenaline in some specific nuclei of the rat brain, using the highly sensitive and specific technique of gas chromatography–mass spectrometry (GC–MS)[7]

In our preliminary experiments, 15 brain nuclei were dissected and assayed for their catecholamine content Male rats (125–175 g) were killed by cervical fracture and the brain rapidly removed and frozen in powdered dry ice The frozen brains were serially sectioned (400 μm, knife angle 30° to the brain axis) in a microtome cryostat (−5° C) and the nuclei located and punched out with a hollow steel tube (0 5–1 2 mm internal diameter), using the atlases of Konig and Klippel[8], Zeman *et al*[9], and Wunscher *et al*[10] as a guide The catecholamines were extracted into 100 μl 0 1 M formic acid 50 mM ascorbic acid, 60 μl was taken to dryness in the presence of internal standards and the dry residue reacted with pentafluoropropionic anhydride[7] After 30 min reaction at 60° C, the excess acylating reagent was removed, and the acylated catecholamines redissolved in 10 μl ethylacetate and 2 μl of this solution was analysed by GC–MS (LKB 9000) using a column packed with 3% SE 54 on Chromasorb G By using the combined instrumentation of GC-MS it is possible to quantitatively measure the catecholamines without having any interference from other compounds The catecholamines are initially resolved on the GC, for example, the retention times for noradrenaline, adrenaline, dopamine and epinine are 370, 425, 510 and 600 s respectively At the retention time for adrenaline (425 s) the MS is focused at m/e 190 which is the base peak (100%) for adrenaline Since the fragment m/e 190 is approximately 60% of the total ions produced, the sensitivity of the detection is extremely high, less than 10 fmol (10×10^{-15}) adrenaline can be measured

Fifteen different rat brain nuclei were initially (triplicate determinations) analysed individually for their catecholamine content Of these regions all contained noradrenaline while only five contained adrenaline The five regions which contained adrenaline were the ventral lateral N reticularis (A_1), the cerebellum at the level of the genu N facialis, including the region from the lateral edges of the iv ventricle, juxtaposed to and possibly including the N superior and N principalis vestibularis and fibrae vestibulo cerebellares, the N locus coeruleus, the habenulu region and the periventricular area of the anterior hypothalamus including the nuclei and regions listed in Table 1 In these five regions (Table 2) the percentage adrenaline ranged from 1 0 to 6 5 of the noradrenaline The highest concentration of adrenaline, 5 7 pmol mg^{-1} protein was found in the periventricular area of the hypothalamus, while the highest adrenaline concentration (as % noradrenaline) was found in the cerebellum

The periventricular areas of the hypothalamus were further dissected into four subregions and assayed for adrenaline Adrenaline was found in all four regions with the highest concentrations being found in the N periventricularis hypothalami, and the area infundibulum and nucleus arcuatus (Table 1)

Table 1 Adrenaline concentrations in the periventricular areas of the hypothalamus

	Adrenaline (pmol mg^{-1} protein)
N Paraventricularis (filiformis) pars magnocellular	2 0 ± 0 24
N Paraventricularis (filiformis) pars parvocellularis	1 6 ± 0 18
N Periventricularis (hypothalami)	4 0 ± 0 40
Area Infundibulum + N Arcuatus	3 4 ± 0 95

*Mean ± s e of three determinations

Table 2 Adrenaline concentrations of five rat brain nuclei and areas*

Area or nuclei	Adrenaline (pmol mg^{-1} protein)	Noradrenaline (pmol mg^{-1} protein)	$\frac{\text{Adrenaline}}{\text{Noradrenaline}} \times 100$
N Reticularis lat (A_1)	1 3 ± 0 33	75 ± 15	1 7
Cerebellum	1 1 ± 0 03	17 ± 4 4	6 5
N Locus coeruleus	1 4 ± 0 16	140 ± 20	1 0
Habenulu	1 2 ± 0 16	57 ± 3 1	2 1
Periventricular area of the hypothalamus	5 7 ± 1 1	160 ± 23	3 6

*Mean ± s e of four determinations

These preliminary data support the proposal of Hokfelt *et al*[11] that a neuronal network containing adrenaline is present in rat brain They base this conclusion on their immunocytochemical localisation of phenylethanolamine-N-methyltransferse (PNMT) in neurones They reported a total of 17 areas or nuclei in rat brain which gave a positive PNMT reaction We have not examined all of these areas as yet but some of our preliminary findings reported here agree with the results of the PNMT reaction, N reticularis lat (A_1) contains cell bodies reacting to PNMT and the locus coeruleus and periventricular areas of the hypothalamus contained PNMT positive terminals Saavedra *et al*[12] have recently reported on the biochemical measurement of PNMT activity in rat brain nuclei The PNMT activity was unevenly distributed and the biochemical localisation of the enzyme agreed well with the histological data of Hokfelt *et al* There does not, however, seem to be good agreement between the PNMT activity and adrenaline concentrations, that is A_1 region 41 5, and N paraventricularis 15 0, PNMT activity against adrenaline concentration of 1 3 and 4 0 pmol mg^{-1} protein, respectively

In these preliminary experiments adrenaline has been measured in a few discrete areas of the rat brain, and found to be present in only certain regions At this point we cannot postulate that the adrenaline is confined to the PNMT neuronal network, additional studies are presently being done to test this hypothesis It is likely, however, that adrenaline is contained in neurons which presumably function independently of the noradrenaline and dopamine neuronal systems

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Effect of reserpine on cationic contents of rat brain

PREPARATIONS of the plant *Rauwolfia serpentina* have been used in India for centuries as a calming drug Reserpine is the principal alkaloid of this plant and its effect on the central nervous system (CNS) has been studied extensively The most important biochemical effect of reserpine on the CNS found *in vivo* is the release of noradrenaline, 5-hydroxytryptamine and dopamine from the amine storage sites in brain so that they are more susceptible to degradation by monoamine oxidase[1] In synaptosomal preparations, reserpine has been shown to inhibit the uptake of noradrenaline and 5-hydroxytryptamine[2] Prolonged reserpine treatment depletes these amines from the brain[1] Amine depletion is thought to be due to changes in the permeability of neuronal membranes[3]

Tetrodotoxin and local anaesthetics increase the glycolysis of cerebral cortex slices[4] and this prompted us to investigate a number of neurotropic agents including reserpine Studies with reserpine revealed that this drug can cause an increase in the potassium content of cerebral tissue both *in vitro* and *in vivo* In view of the important role played by cations in the regulation of brain metabolism[5], the effect of reserpine on the potassium contents is of great interest and we are surprised that such measurements have not been made previously

Rat cerebral cortex slices were prepared and incubated as described earlier[4,5] and anaerobic glycolysis, cationic contents, ^{22}Na transport and respiration were measured For the *in vivo* experiments rats were given reserpine (2 mg kg^{-1}, Ciba) or saline intraperitoneally for 5 d after which they were killed, the brains quickly removed, weighed and protein-free extracts prepared for cation analysis by a Coleman flame photometer

The *in vitro* experiments, details of which will be published elsewhere, showed that during anaerobic conditions, 12-120 μM reserpine greatly enhances the rate of glycolysis by adult rat and guinea pig cerebral cortex slices The maximum effects observed were up to 3–4 times the normal rate It was thought possible that reserpine may exert this effect indirectly by amine release Experiments carried out with biogenic amines showed that unlike reserpine, these amines have no significant effect on anaerobic glycolysis It was therefore concluded that reserpine may also exert its effect on the anaerobic glycolysis by increasing K$^+$/Na$^+$ ratio, as is the case with tetrodotoxin and local anaesthetics This was found to be true and reserpine had a significant effect in increasing the K$^+$ contents of the cerebral cortex slices incubated under anoxic conditions, the increase being up to 34 μeq per g initial wet weight of the tissue as compared to 17 μeq per g initial wet weight for the controls

Other experiments further demonstrated that 12 μM reserpine may affect the cationic movements It was found that the respiration of rat cerebral cortex slices, stimulated by 10 μM protoveratrine, was suppressed by 12 μM reserpine These effects were much more evident in a Ca^{2+}-free Krebs–Ringer phosphate medium ^{22}Na movement was also suppressed by reserpine in these conditions

A most interesting effect of reserpine was observed *in vivo* (Table 1) It was found that chronic reserpine administrations results in an increase in the K$^+$ contents of brain of more than 25% while the Na$^+$ contents are not affected The cationic contents described here were determined in the whole brain It is possible that most of the K$^+$ increase occurs intracellularly (and primarily in the neurones as compared to the glia), since very little extracellular space is present in brain

Table 1 Effect of chronic reserpine administration on the Na$^+$ and K$^+$ contents of rat brain

Treatment	K$^+$ (μeq per g fresh weight)	Na$^+$ (μeq per g fresh weight)
Control (12)	122 7 ± 5 7	48 5 ± 3 0
Experimental (12) (Reserpine, 2 mg kg^{-1} d^{-1} for 5 d)	157* ± 4 7	49 3† ± 2 5

K$^+$ and Na$^+$ contents were determined as given in the text Values obtained, and their standard deviations are given Number of observations are shown in parentheses

*P < 0 001, highly significant

†P > 0 4, not significant

The effect of reserpine on the amine uptake presumably results from changes in the permeability of neuronal membrane with a concomitant increase in the steady state level of K$^+$ Our results have wide implications Sedation induced by reserpine is thought to result from a decrease in the quantity of monoamines[1] High cerebral potassium in reserpinised animals may cause neuronal hyperpolarisation resulting in the potentiation of sedative effects It has been argued that barbiturates and other sedatives may primarily act by inhibiting impulse conduction rather than amine metabolism[7]

Our observations also raise the question whether an increase in the level of cerebral K$^+$ is the primary effect of reserpine or is caused indirectly due to other central effects The former possibility seems to be true The uptake of amines by nerve encings is dependent on the presence of Na$^+$ (ref 8), and the mechanism is activated by excitation and thus depolarisation[10] Membrane affinity for neurotransmitters is affected by nervous stimulation[9] Moreover, K$^+$ has been shown to compete with Na$^+$ for the Na$^+$-dependent uptake of monoamines[10,11] Almost 80% of noradrenaline released at the synapse is inactivated by the uptake mechanism[9] It seems that the increase in neuronal K$^+$ affects the uptake of amine by storage granules, presumably by competing with Na$^+$ (refs 10 and 11) Modulation of membrane affinity to neurotransmitters, therefore, seems to be involved in the action of reserpine Further investigations are in progress to examine these possibilities

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S-100 protein in synapses of the central nervous system

THE brain-specific S-100 protein[1] is predominantly localised in glial cells in the central nervous system and is synthesised by glial cells in tissue culture[2-5] S-100 is, however, also present in neurones[6-9], located in both the neuronal perikaryon and the nucleus[6,9-11] It has been proposed that the protein is transported along the axon[10] although the presence of S-100 in the Schwann cell[12,13] calls for confirmation of an intra-axonal localisation of the protein Approximately 90% of the S-100 in the brain is easily soluble and extracted by water[14-15], but an additional small amount can be extracted from the particulate matter with *n*-pentanol. Rusca *et al.*[14] showed a further release 5–7% of the S-100 from whole beef brain, and Haglid *et al.*[13] extracted 7% from human grey matter and 3% from white matter with n-pentanol. The percentage of total S-100 which remained bound to the particulate matter after water extraction was considerably higher in neuronal perikarya than in glial cells (manuscript in preparation). We have investigated the ultrastructural location of the relatively large amounts of membrane-bound neuronal S-100.

White albino rabbits, weighing 1.5–2 kg, were killed after mebumal anaesthesia and decapitated. Parts of the CNS were dissected fresh, immersed in ice-cold 0.1 M cacodylate buffer, *p*H 7.4, and cut to 10–20 μm thick sections on a Vibratom (Oxford Instrument). In other experiments, the brains were fixed by intra-aortal perfusion with 2% formaldehyde in 0.1 M cacodylate buffer, *p*H 7.4, and rinsed in the same buffer after a fixation time of 5 min. All solutions in contact with the tissue slices contained 2.5 mM EDTA or EGTA. The fresh and prefixed sections were incubated for 30 min in 0.1 M cacodylate buffer, containing antiserum against S-100 coupled with peroxidase. They were then extensively rinsed, and thereafter fixed in 2.5% glutaraldehyde and 2% formaldehyde in cacodylate buffer.

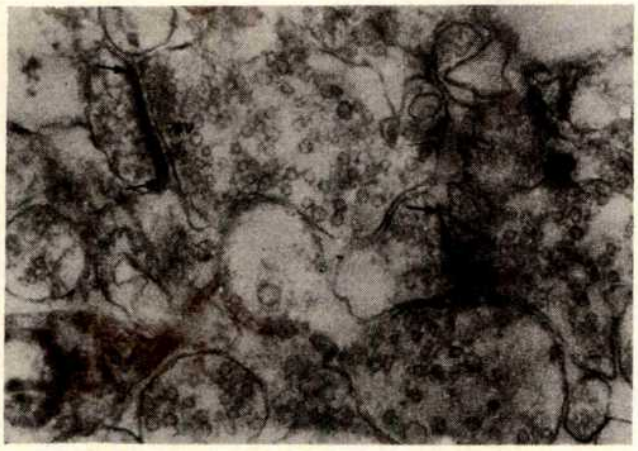

Fig. 1 Electron micrograph of synapses in the frontal cortex of an adult albino rabbit. The tissue piece was treated with rabbit antiserum to beef S-100 protein coupled to peroxidase as described in the text. The postsynaptic membranes (arrow) show a strong peroxidase activity. No activity is seen in the synaptic cleft or in the synaptic vesicles. The activity of the presynaptic membrane is about the same as in other neuronal membranes. × 42,750.

Cerebellar glomerular complexes were isolated by Ficoll gradient centrifugation according to A.H., H.-A.H., and Å. Sellström (in preparation). The glomerular suspension was incubated at room temperature for 10 or 30 min with the peroxidase-coupled antiserum against S-100, sedimented and treated like the tissue sections. All material was then thoroughly rinsed and incubated in a cacodylate buffered solution of 3.3-diaminobenzidine and hydrogen peroxide[16]. The samples were rinsed, postfixed in osmium tetroxide, dehydrated and embedded in Epon. Thick sections were cut on an ultramicrotome (LKB Ultrotome III) and examined in a phase contrast microscope. Selected areas were sectioned for examination in an electron microscope (Siemens 1 A). They were either unstained or double-stained with uranyl acetate and lead citrate. The purification of beef S-100 and the production of anti-beef S-100 rabbit antiserum, as well as the monospecificity of the antibodies in the serum were tested by microcomplement fixation, immunoelectrophoresis and other electrophoretic methods[13,15]. The peroxidase (Sigma Type VI) was coupled to the antiserum according to Avrameas[17]. The peroxidase-coupled anti-beef S-100 rabbit serum was separated from free peroxidase by G-75 Sephadex column chromatography. The specificity of the peroxidase-coupled anti-beef S-100 rabbit serum reactions was checked by the use of a control antiserum which had been repeatedly (12 times) absorbed with purified beef S-100 protein. Test and control antisera were diluted and treated in an identical manner. The completeness of the absorption was tested by microcomplement fixation against the original antigen. The final dilution of antiserum was titrated by chessboard until maximal test–control difference in peroxidase activity was observed.

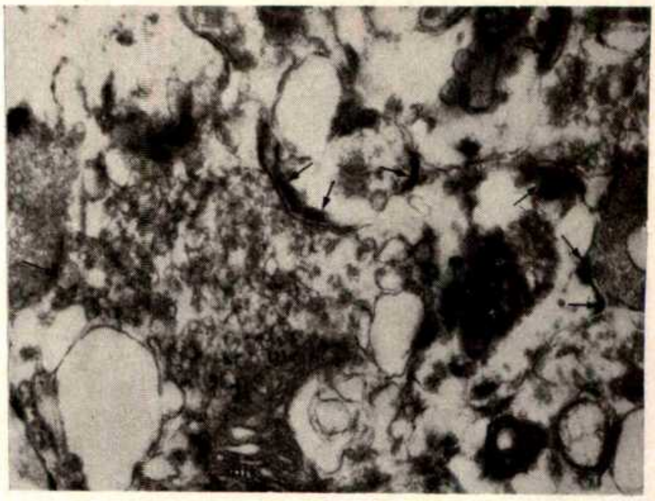

Fig. 2 Electron micrograph of an isolated cerebellar glomerulus after treatment with rabbit antiserum to beef S-100 protein coupled with peroxidase. Note the dark reaction products indicating peroxidase activity, that is, localisation of S-100 protein at the postsynaptic membrane (arrow). The other neuronal membranes have a lower level of activity than the postsynaptic membrane. The mitochondria (mit) may show nonspecific activity. × 31,950.

Specific peroxidase activity was seen in most synaptic regions of the cerebral and cerebellar cortex. Heavy precipitates were localised exclusively on, and immediately interior to, the postsynaptic membrane (Fig. 1). No activity was seen in the synaptic cleft. All other parts of the neuronal membrane—including the presynaptic membrane —had a low or moderate but specific peroxidase activity. The synaptic vesicles, endoplasmic reticulum, filaments, microtubules and nuclei did not show any significant peroxidase activity. Some mitochondria showed peroxidase activity also after treatment with antisera absorbed repeatedly with the antigen protein S-100. Such nonspecific peroxidase activity has previously been described[17]. Neither peroxidase-coupled rabbit antisera directed against other soluble or particulate brain proteins, nor free peroxidase showed any localisation to the postsynaptic membrane.

In order to investigate whether the distribution of peroxidase activity was affected by penetration barriers, we examined suspensions of isolated cerebellar glomeruli, which consist of clusters of synaptic contacts. Again, intense peroxidase activity was observed at the postsynaptic membrane (Fig. 2). A different pattern was observed in the synaptic terminals of the rods and some bipolar nerve cells in the rabbit retina (Fig. 3). An intense reaction was seen in the synaptic ribbon and in both the pre- and postsynaptic membranes in these composite synaptic terminals. In retinal synapses of more conventional type the peroxidase reaction was confined to the postsynaptic region, as in the cerebral and cerebellar synapses.

The S-100 protein visualised by peroxidase-coupled anti-beef S-100 rabbit serum is presumably particulate bound, as fixed and fresh slices gave a similar picture—the fresh slices were subjected to buffer solution in multifold excess which makes the soluble S-100 protein easily diffusable. The

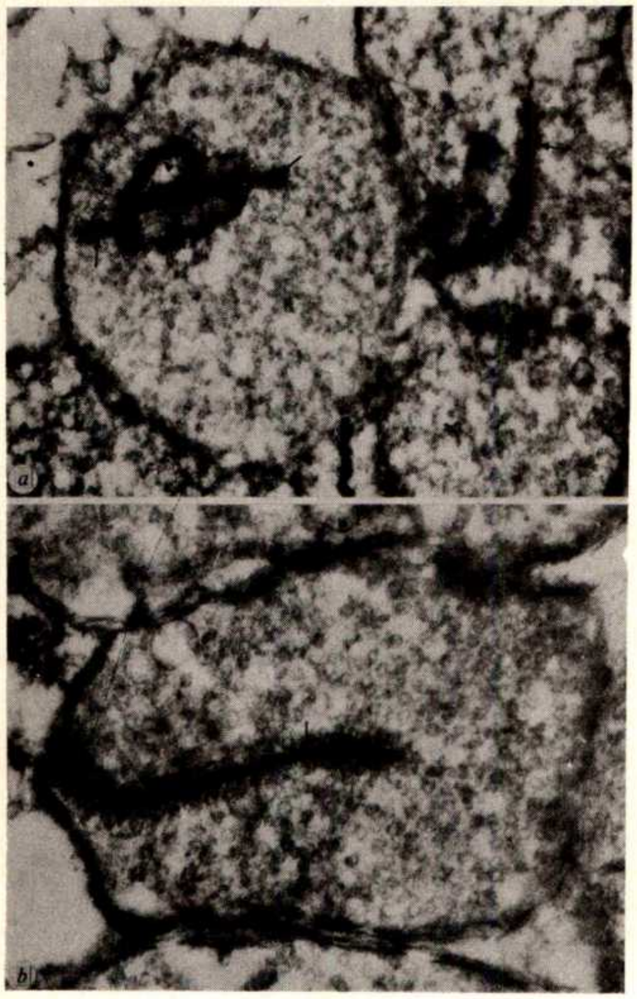

Fig. 3 Electron micrograph of synaptic terminals in the outer plexiform layer of the rabbit retina treated as in Fig. 1. Dark deposits, indicating peroxidase activity, are seen in the synaptic ribbon (arrow) and at the synaptic contacts with bipolar nerve cells (arrowhead) and also, although at a lower level, in the neuronal membrane. The synaptic vesicles show mainly background activity. *a*, × 21,300; *b*, × 22,720.

soluble form of S-100 does not bind the subcellular particulate material in the presence of EDTA (B. W. Moore, communicated at NATO Advanced Study Institute meeting in Cortona, Italy, 1971).

The functional role of the S-100 protein is so far unknown. Its interaction with Ca^{2+} ions[18] and with the passage of monovalent and divalent cations across artificial lipid membranes[19] may lead to speculations about its function. We here present data which indicate a specialised synaptic localisation of the S-100 protein. This might imply a functional interrelation between a glial brain-specific protein and synaptic function, since so far there is no evidence that this protein is synthesised in neurones.

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Dihydrostreptomycin accumulation in *E. coli*

DIHYDROSTREPTOMYCIN is an antibiotic composed of streptidine, dihydrostreptose and N-methyl-L-glucosamine joined by two glycosidic bonds. It differs from streptomycin only in that the aldehyde group on the middle residue (streptose) is reduced. Both compounds have similar biological actions[1,2] but their accumulation by bacterial cells has been little studied. Passive diffusion is often assumed to be responsible, although Mitchell thought it improbable that a molecule such as streptomycin passes through a lipid layer[3]. Anand *et al.* proposed a mechanism in two phases[4]. First, a small amount of antibiotic would enter by passive diffusion regardless of an intact lipid layer and affect protein synthesis so as to cause deterioration of membrane integrity. Then more rapid accumulation would follow by passive diffusion through the disrupted lipid barrier. Alternatively, Hurwitz *et al.* showed streptomycin accumulation to be sensitive to chloramphenicol during initial exposure and insensitive after several minutes of exposure. They suggested facilitated diffusion, with entry of the antibiotic depending on a streptomycin-induced protein[5].

Carlson and Bockrath found dihydrostreptomycin accumulation to vary depending on whether bacterial cells grew in a glucose-defined medium or in nutrient broth[6]. Accumulation was roughly fifty times more efficient in nutrient broth cultures or with cells recently transferred from nutrient broth in the defined medium. After an apparent lag of a few minutes, the kinetics of accumulation were generally linear with time for at least 30 min. Anand *et al.* used cells growing in defined medium and Hurwitz *et al.* used cells growing in nutrient broth. We have repeated the observation of a chloramphenicol-insensitive phase of accumulation and have found that cells growing in nutrient broth accumulate antibiotic during this phase by a transport mechanism involving a positive contribution from cellular metabolism.

A culture of *Escherichia coli* **B** growing exponentially

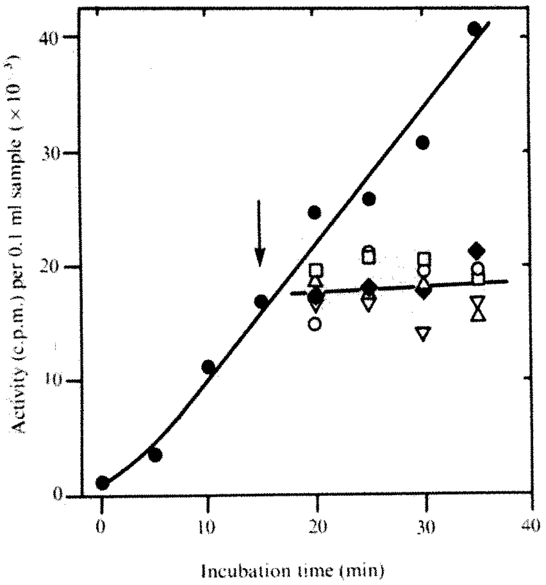

Fig. 1 Inhibition of ^{3}H-dihydrostreptomycin accumulation. ^{3}H-dihydrostreptomycin sesquisulphate (2 μg ml^{-1}, Amersham/Searle, specific activity 2.1 mCi mg^{-1}) was added at zero time to an exponentially growing culture of *E. coli* B in Difco nutrient broth (4.8×10^7 bacteria ml^{-1}). At 15 min (arrow) aliquots were removed to separate cultures for specific perturbations during continued incubation: ▽, transferred to ice bath; ◆, potassium arsenate (10 mM); △, mercuric chloride (0.2 mM); □, N-ethyl maleimide (1 mM); and ○, iodoacetate (2 mM). All incubations were at 37° C (except ▽) with aeration. Accumulated radiolabelled antibiotic was determined during incubation time: 0.1 ml samples were diluted × 100 into buffer[6] at room temperature, filtered on to glass fibre filters, rinsed twice with 10 ml of buffer, dried and quantitated by liquid scintillation counting.

in Difco nutrient broth was challenged at zero time with ^{3}H-dihydrostreptomycin (^{3}H-dhSm, 2 μg ml^{-1}). Chloramphenicol (20 or 200 μg ml^{-1}) inhibted ^{3}H-dhSm accumulation when added at zero time but had little effect if added 10 min later. The rate of accumulation when chloramphenicol was added at zero time (just before the addition of ^{3}H-dhSm) was very small and showed no tendency to increase. Repeated experiments found chloramphenicol to be a good inhibitor if added at zero time, a moderate to good inhibitor of accumulation if added 5 min after the initial exposure to ^{3}H-dhSm, and moderate to poor if added after 10 min. Therefore, it seems that protein synthesis is of marginal importance to the accumulation of ^{3}H-dhSm by *E. coli* B once the cells have been exposed to dihydrostreptomycin for a period of 10 min.

Table 1 Perturbations of ^{3}H-dihydrostreptomycin accumulation

Challenge	Concentration (mM)	Accumulation (%)
Ice bath	—	0
Dinitrofluorobenzene	0.5	0
2,4-Dinitrophenol	2.0	0
Potassium cyanide	0.5	5
Mercuric chloride	0.2	8
Potassium arsenate	10.0	8
Iodoacetate	2.0	12
N-ethyl maleimide	1.0	21
Phenalzine methyl sulphate	0.1	22
Potassium fluoride	5.0	47
Sodium fluoride	5.0	58
Amytal	0.1	58
Rotenone	0.1	79
Oxamic acid	0.5	114

Perturbations of ^{3}H-dihydrostreptomycin accumulation were determined as described in Fig. 1. In each case the indicated challenge was introduced after 10 or 15 min of accumulation. The effect is recorded as the slope of accumulation after the challenge relative to the slope of accumulation in the control.

Dramatic inhibition of accumulation may, however, be produced by cold and by several anti-metabolites added 10 or 15 min after the initial exposure to ^{3}H-dhSm. Figure 1 shows several examples of an abrupt cessation of ^{3}H-dhSm accumulation. The perturbations are not introduced until 15 min after initiation of exposure. The results of these experiments and several others are summarised in Table 1. Clearly they suggest a metabolism-dependent mechanism of ^{3}H-dhSm accumulation. Several inhibitors of metabolism can cause accumulation to stop and plateau regardless of a hypothetical deterioration of membrane integrity or a physiological development obviating the requirement for protein synthesis.

Anand *et al.*, who rinsed their samples with water, found toluene treatment of cells to cause an apparent influx of streptomycin (with an exogenous antibiotic concentration of 40 μg ml^{-1}). In our experiments cells growing in nutrient broth accumulated large amounts of ^{3}H-dhSm (from an exogenous concentration of 2 μg ml^{-1}); we rinsed individual samples with a buffer[6] and found the addition of toluene then caused an apparent efflux of the antibiotic (Fig. 2). To ensure that the toluene treatment did not totally dissociate the cell structure or alter the filtration system to allow cells to pass through the filter, cells prelabelled with ^{3}H-uracil were also treated. In Fig. 2 the open symbols show this activity (labelled ribosomes and nucleic acid) to remain on the filter unless the cells were further treated with lysozyme.

An integral cellular membrane is therefore essential in our assay for accumulated ^{3}H-dhSm. Apparently antibiotic molecules which have already entered the cell do not

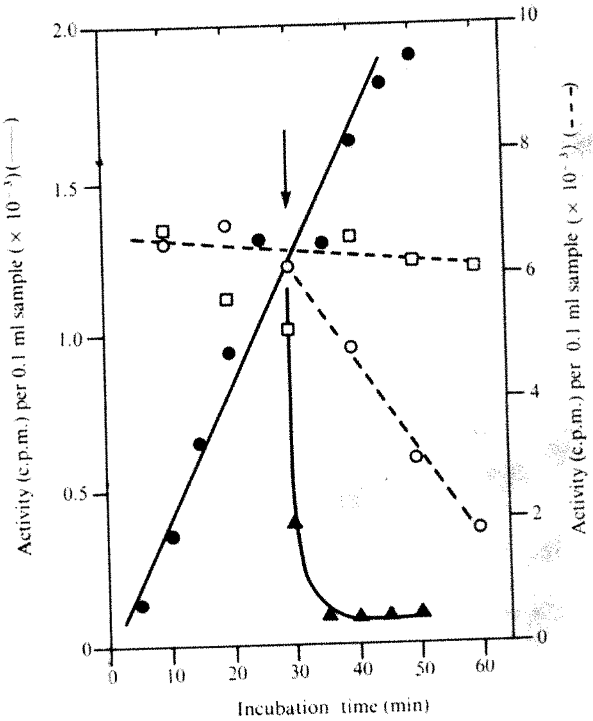

Fig. 2 Effects of toluene on the accumulation assay. ^{3}H-dihydrostreptomycin (2 μg ml^{-1}, Mallinckrodt, specific activity, 0.25 mCi mg^{-1}) was added at zero time to an expo-nentially growing culture of *E. coli* B in Difco nutrient broth (6×10^7 bacteria ml^{-1}). After 30 min 0.02 ml ml^{-1} toluene was added to one aliquot (-▲-), with the remaining culture serving as a control (-●-). A nutrient broth culture of *E. coli* B was also grown with ^{3}H-uracil (1.5 μCi ml^{-1}) for several generations and divided into two aliquots at zero time. Dihydrostreptomycin (unlabelled), 2 μg ml^{-1}, was added to both; one (- -□- -) was treated with toluene, 0.02 ml ml^{-1}, at 30 min, and the other (- -○- -) was treated with toluene after 15 min and lysozyme, 0.4 mg ml^{-1}, after 30 min.

equilibrate rapidly with the rinse buffer unless the membrane is altered by an agent such as toluene. In consequence, it seems the normal membrane is not transparent to ^{3}H-dhSm and a brief period of exposure to ^{3}H-dhSm does not open the membrane.

Plotz *et al.* have shown that when normal cells are held in a water rinse apparent uptake is increased[7]. We found toluene-treated cells, similarly held in a water rinse containing a representative amount of ^{3}H-dhSm, to yield an even larger apparent uptake. We therefore doubt that toluene treatment of cells in culture necessarily allows ^{3}H-dhSm to rush in. More likely, the equilibrium of ^{3}H-dhSm between the medium and the cell volume is principally a function of the cation-exchange capacity of cell biomass[8] and the intracellular concentration of competing cations. The apparent accumulation of antibiotic in toluene-treated cells would vary with the ionic composition of the rinse solution.

In summary, after a period of potentiation during which cellular uptake of ^{3}H-dhSm becomes refractory to inhibition by chloramphenicol, the accumulation of ^{3}H-dhSm is still sensitive to inhibitors of metabolism. Our assay for accumulation of antibiotic uses a buffer rinse which does not seem to remove accumulated ^{3}H-dhSm unless the integrity of the cell membrane is disrupted. These findings are consistent with an accumulation mechanism in *E. coli* for dihydrostreptomycin that involves either active transport or group translocation.

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Maternal inheritance of mammalian mitochondrial DNA

EUKARYOTIC cells contain a class of cytoplasmic DNA molecules which are found only within the mitochondria, where they replicate and are transcribed (for a general review see ref. 1). This mitochondrial DNA (mtDNA) constitutes the 'mitochondrial genome' and carries genetic information essential to mitochondrial function. In view of its extranuclear location, it is not surprising that inheritance of the mitochondrial genome is not governed by the same rules that apply to chromosomal genes. For fungi[2-7] and amphibians[8] there is evidence that the mitochondrial genome of an individual is derived solely from the maternal parent (in contrast to chromosomal genes, which are inherited biparentally). We present here evidence that the mitochondrial genome is inherited maternally in mammals too.

This problem is not readily approachable by the standard techniques of genetic analysis, because mutations in the mammalian mitochondrial genome have not been identified. We have, therefore, used specific cleavage by a restriction endonuclease to look directly at the inheritance of mtDNA nucleotide sequences. When any homogeneous DNA preparation is digested to completion by a site-specific restriction endonuclease, a characteristic set of fragments is produced. If the DNA is of low complexity, as in the case of simple viruses[9,10] or mammalian mtDNA[11], separation of the restriction fragments on the basis of molecular weight by gel electrophoresis produces a characteristic 'fragment pattern' (Fig. 1). This pattern is characteristic of the original DNA molecule in much the same way that the tryptic fingerprint of a protein is characteristic of that particular protein.

We have studied the inheritance of mtDNA by isolating pure mtDNA from hybrids between two closely related mammalian species. The two parental mtDNAs are readily distinguishable by restriction enzyme cleavage analysis; analysis of the mtDNA from the hybrid animals yielded a fragment pattern characteristic of the maternal species. None of the DNA fragments specific for the paternal species were observed.

We obtained our material from common equine species: the horse (*Equus caballus*), the donkey (*E. asinus*), and the two reciprocal hybrids between them, the mule and the hinny. The two hybrids are defined as (1) ♀ horse × ♂ donkey → mule and (2) ♀ donkey × ♂ horse → hinny. Mules and hinnies may be of either sex but are generally sterile.

Mitochondria were partially purified from liver or heart tissue, and the DNA extracted from such preparations was centrifuged in CsCl-ethidium bromide gradients[12]. Pure mtDNA was isolated from the supercoil band in such gradients. The purified mtDNAs were then digested with a restriction endonuclease isolated from *Haemophilus aegyptius*, endonuclease Z[13] (endonuclease R·Hae III in the nomenclature of ref. 14), and the resulting digests were analysed by electrophoresis in 3.5% polyacrylamide gels. The DNA-containing bands were demonstrated by fluorescence after staining with ethidium bromide[15].

The fragment patterns obtained from horse and donkey mtDNAs are clearly distinguishable (Fig. 1). We examined DNA from six horses, two full-sized (one of each sex) and four ponies. Their mtDNA cleavage patterns were almost identical. The two horse patterns were indistinguishable, and the pony patterns differed in the position of one or two of the lower molecular weight fragments. These minor intraspecies variations in the fragment pattern seem to be significant and will be discussed elsewhere (manuscript in preparation). mtDNA from two male donkeys yielded fragment patterns indistinguishable from each other, but clearly different from the characteristic horse pattern. But although the two species yield readily distinguishable fragment patterns, there appears to be considerable homology between the two patterns.

Cleavage analysis of mule mtDNA gives the maternal (horse) fragment pattern (Fig. 1). All three animals that we have examined (one male, two females) yielded the standard horse pattern. This could be explained in terms of a general phenomenon of maternal inheritance of the mtDNA. Alternatively, however, it could be a result of a species-specific loss of donkey mtDNA in horse–donkey hybrid cells. To distinguish between these possibilities we have analysed the mtDNA from a hinny. Again the maternal pattern was observed (a donkey pattern in this case, see Fig. 1). The liver of this female hinny contained predominantly horse glucose-6-phosphate dehydrogenase (G6PD), but only donkey mtDNA (Fig. 2). This proved that the animal contained horse chromosomes (G6PD is X linked), and verified the word of the breeder that the animal was, in fact, a hinny. The preponderance of horse G6PD in this hybrid suggests the preferential inactivation of the maternal donkey X chromosome in this animal and is consistent with certain published reports of non-random X-chromosome inactivation in mules and hinnies (ref. 16 and review in ref. 17). It should be noted that a model consisting of an X-linked template for mtDNA synthesis, which is non-randomly inactivated in the hybrid animals, is not a reasonable explanation for the observed maternal inheritance of mtDNA since in this hinny it is the

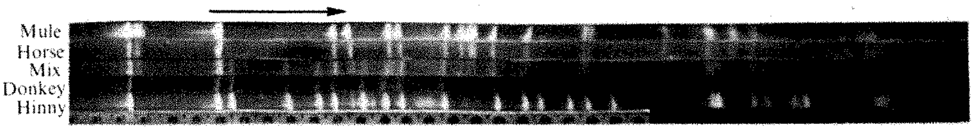

Fig. 1 Polyacrylamide gel electrophoresis of restriction fragments of equine mtDNAs. The arrow indicates the direction of electrophoresis. Reading from the ruler, the samples are: (1) hinny heart; (2) donkey liver; (3) mixture of donkey liver and horse liver; (4) horse liver; (5) mule liver. Analysis of liver mtDNA from the hinny gave a pattern the same as shown here for heart mtDNA. Our preparation of mtDNA will be described in detail elsewhere (manuscript in preparation). Tissue was homogenised to disrupt cells, then nuclei and debris were sedimented in the presence of 0.25 M sucrose at 500*g* for 10 min. After recentrifuging the supernatant at 500*g*, the mitochondria were pelleted twice at 10,000*g* for 10 min. The pellet was suspended in 0.15 M NaCl, 0.05 M EDTA, 0.01 M Tris, *p*H 7.4, lysed by the addition of 2% sodium dodecyl sulphate (SDS), phenol extracted twice, and then dialysed against SSC. About 20 ml of this mitochondrial nucleic acid preparation was then sedimented into an 18 ml preformed CsCl-ethidium bromide gradient (ρ = 1.4 to 1.7; 100 μg ml^{-1} ethidium bromide) poured in a 1 × 3.5 inch cellulose nitrate tube. The gradient was centrifuged 18–24 h at 22,000 r.p.m. in a SW27 rotor. DNA bands were demonstrated by fluorescence in ultraviolet light, and the bottom (supercoil) band was collected, freed of ethidium bromide by isopropanol extraction, then dialysed against SSC. The mtDNA was pelleted at 33,000 r.p.m. for 10 h in a SW50.1 rotor, the supernatant was discarded, and the mtDNA was dissolved in 0.15 M NaCl, 0.01 M Tris, *p*H 7.5. Yields from this type of preparation range from about 0.1 to 1.0 μg of supercoiled mtDNA per gram of tissue. To 45 μl of mtDNA solution (containing 10–20 μg DNA) were added 10 μl 0.13 M $MgCl_2$, 5 μl 0.02 M β-mercaptoethanol, and 5 μl endonuclease Z (enough enzyme to give a limit digest of 50 μg of SV40 DNA). Digestion was carried out at 37° C for 10 h, solid sucrose was added to 20% and the sample was layered on top of a cylindrical 3.5% polyacrylamide gel (gel composition as in ref. 9 except that agarose was omitted) 7 mm in diameter and about 50 cm in length. Electrophoresis was carried out for about 18 h at 310V, at which time bromophenol blue added to the sample is near the bottom of the gel. Gels were removed gently from the plastic tubes by air pressure into electrode buffer[9] containing 1 μg ml^{-1} ethidium bromide, and stained at room temperature for 2 h. Gels were placed against a black background, illuminated at a distance of a few inches with a Sylvania type BLB fluorescent black-light bulb and photographed at *f*4.7 for 60 to 120 s on Polaroid type 55 P/N film. An ultraviolet filter and an orange filter approximating the colour of the DNA-ethidium fluorescence were placed in front of the camera lens. These fragment patterns are interpretable in terms of a single type of mtDNA molecule of approximately 1 × 10^7 daltons for each of the two equine species. A quantitative analysis and comparison of these patterns will be presented elsewhere.

paternal X which is active. In summary, the evidence presented here demonstrates that the mtDNA of both mules and hinnies is maternally derived. We believe that as little as 5% paternal mtDNA would have been detectable in our experiments; however, we cannot exclude the presence of smaller amounts of paternal mtDNA. It seems reasonable to assume that the experiments look directly at inheritance of the primary nucleotide sequence of the mtDNA, however, we cannot logically exclude the possibility that factors which modify cleavage sites on the mtDNA differ between the two species and are maternally inherited.

Since the mtDNA is maternally inherited in both reciprocal hybrids, it seems reasonable to conclude that mtDNA is maternally inherited in each of these equine species. It would be surprising if this phenomenon were restricted to equine mammals. These experiments support the idea that the mitochondrial genome consists of cytoplasmically replicating mtDNA which is not the product of any chromosomal template.

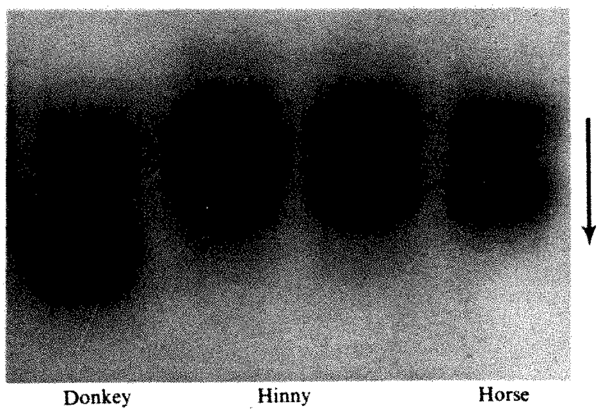

Fig. 2 Starch gel electrophoresis of equine G6PDs. Liver extracts were electrophoresed by the Tris-EDTA-borate method and stained for G6PD activity[25]. The left channel contains donkey G6PD, the two middle channels are identical samples of female hinny G6PD, and the right hand channel is horse G6PD. An arrow indicates the direction of electrophoresis. It has been suggested[26] that the slower moving (minor) components in both horse and donkey G6PD represent degradation products. The hinny samples are clearly predominantly horse G6PD, but appear possibly to contain a trace of the faster moving donkey enzyme.

Although it is not surprising that mtDNA is inherited maternally in mammals it could not be predicted *a priori* since the middle piece of the mammalian spermatozoon, containing the mitochondria, usually enters the egg[18]. In the mouse, mitochondria from the sperm have been observed to scatter in the cytoplasm of the egg[19,20]. In the light of these observations at least two mechanisms for maternal inheritance can be proposed: (1) the paternal (spermatozoon) mitochondria may be incapable of replication during development of the fertilised eggs, (2) maternal inheritance may result from a quantitative preponderance of maternal (egg) mitochondria in the zygote. It is amusing to consider some numerological consequences of the second type of mechanism. It has been estimated that a bull sperm contains 72 mitochondria[21]. Mammalian eggs are known to be rich in mitochondria[18,22], and if the concentration of mtDNA is similar to that found in the cytoplasm of echinoderm eggs[23,24], an equine egg would contain about 10^6 molecules of mtDNA. This would imply that if paternal and maternal mitochondria replicate at equal rates, then only about 10^{-4} of the mtDNA in a mammal would be paternally derived. This would be below the limit of detection of our experiments. Furthermore, the sperm mitochondria could be partitioned into cells during the formation of the blastocyst, before the onset of mitochondrial replication. As a consequence, many clones of cells in the developing embryo may be completely free of paternal mtDNA, even if paternal mitochondria are viable. It therefore seems unnecessary to postulate any replicational defect in the paternal mitochondria to account for the observed maternal inheritance of mtDNA. The possibility still exists that paternal mitochondrial genomes are present in very small numbers in mammalian tissues, and that sufficiently sensitive experiments could detect their presence.

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T3 x T7 phage crosses leading to recombinant RNA polymerases

THE related virulent bacteriophages T3 and T7 code for RNA polymerases which are specific for promoters located on the DNA of these phages[1,2]. These single peptide chain polymerases—the products of gene 1 (refs 1 and 3)—are genetically homologous but can be distinguished by their template specificity and their molecular weight. The T3 RNA polymerase transcribes T7 DNA at only about 10% of the rate at which T3 DNA is transcribed, and the T7 RNA polymerase transcribes T3 DNA at about 60% the homologous rate[2]; and, by direct comparison with the T7 polymerase, the molecular weight of the T3 polymerase was found to be about 5% lower than that of the corresponding T7 protein[4,5] (whose molecular weight has been estimated at 107,000 (ref. 1), 110,000 (ref. 2), 100,000 (ref. 6), and 108,000 (ref. 4). Since phages T3 and T7 can be crossed[7], the differences between the T3 and T7 RNA polymerases provide a model system for studies on the correlation between genetic constitution and functional specificity. Here we report some initial experiments in this direction.

The procedure was to isolate genetic recombinants within gene 1 of T3 and T7, and to analyse the corresponding gene 1 products with regard to template specificity and molecular weight. Since the recombination frequency in crosses between T3 and T7 is very low[7,8], parental types carrying two amber mutants were chosen to avoid *am*+ revertants. One parent was T7 *am*H13.*am*H280 (with both *am* markers within gene 1) and the other was T3 *am*H222.*am*H283 (a gene 1–gene 3 double mutant). The exact locations of the mutational sites are shown in Fig. 3. The proportion of *am*+ recombinants among the progeny of such crosses was $1-4\times10^{-5}$; that is, about a thousand times less than in corresponding crosses with homologous phage. From such a T3×T7 cross we picked ten *am*+ plaques for further analysis.

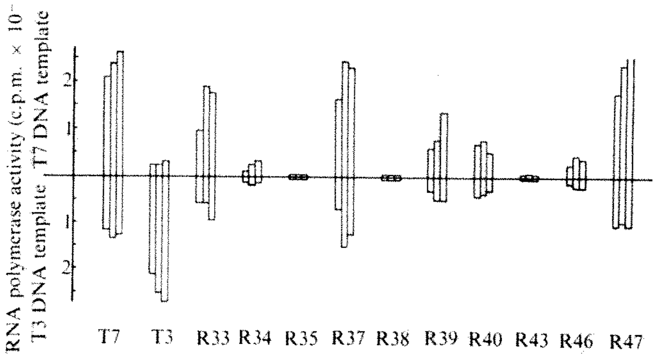

Fig. 1 RNA polymerase activity in cell-free extracts of phage-infected host bacteria, assayed with T3 or T7 template DNA. Exponentially growing cells of *E. coli* B (2×10^8 cells ml^{-1}) in casaminoacids medium[8] (37° C) were infected with T7, T3 or various genetic recombinants between T3 and T7 (multiplicity: 10 PFU/cell). At 5, 7 and 9 min after infection (first, second and third column, respectively), sample (10 ml) of each culture was poured on ice with chloramphenicol (300 μg ml^{-1}, final concentration) and centrifuged immediately. The pellets were resuspended in 0.5 ml buffer (0.05 M Tris-Cl, *p*H 8; 0.01 M 2-mercaptoethanol; 2 mg ml^{-1} bovine serum albumin) and the cells were disrupted by low intensity ultrasound (Branson sonifier B-12, microtip, 0° C). The extracts were centrifuged and the supernatant fluid was assayed at 37° C for rifampicin-resistant RNA polymerase activity as described by Chamberlin *et al.*[1], except, that first, 100 μl of assay mixture contained 7 μg of T3 or T7 DNA and second, 20 μl of cell extract were added to 100 μl of assay mixture. The RNA polymerase activity is here given as c.p.m. of ^{14}C-UTP (500 c.p.m. nmol^{-1}) incorporated into acid precipitable material, per assay.

The first step in this analysis aimed at ascertaining the recombinatorial origin of these *am*+ stocks. For this, two non-selected markers were checked: first, the ability to code for an active *S*-adenosylmethionine-cleaving enzyme (AdoMet-ase), which depends on the T3-specific *sam*+ gene (ref. 3), and second, the specificity towards T3 and T7 antiserum, determined by the product of gene 17 (serum blocking protein)[9]. (The *sam*+ gene is located at the 'left' end of the genetic map[3] and gene 17 lies at about 0.9 genome length, that is, near the 'right' end of the map[10].) The assay techniques for these markers were described elsewhere (refs 11 and 9). All the ten *am*+ stocks were *sam*+ and had the antiserum specificity of T7. Thus, these phages were undoubtedly recombinants between T3 and T7.

These T3×T7 recombinants were then analysed with regard to the activity and template specificity of the phage RNA polymerase they induce after infection of *Escherichia coli* B. As shown in Fig. 1, all the recombinant RNA polymerases either had a preference for T7 DNA or had virtually no measurable *in vitro* activity. The recombinants with no activity at 37° C (R35, R38, R43) were also grown and tested at 25° C, with the same result. Since the phage RNA polymerase is essential for phage growth, as evidenced by the fact that amber mutants in gene 1 cannot grow on su^- hosts, it must be concluded that the RNA polymerases coded by these recombinants are functionally active *in vivo* but are so unstable that they become inactive in the process of extraction. The recombinant RNA polymerases active *in vitro* were also less stable than the wild type enzymes, as shown by a higher temperature sensitivity.

Next, the recombinant RNA polymerases were characterised with regard to their molecular weights. This was done by polyacrylamide gel electrophoresis, in the presence of SDS, of extracts of host cells infected with phage and supplemented with ^{35}S-methionine, followed by autoradiography of the dried gels. This method (successfully used to characterise the T7-coded intracellular proteins[6,12]) revealed that the RNA polymerases of three of the tested recombinants had the molecular weight of the T3 RNA polymerase while the others—with one exception—had the molecular weight of the corresponding T7 enzyme (Fig. 2). The one exception, R43, coded for a gene 1 product with a molecular weight intermediate to those of the RNA polymerases of T3 and T7 (Fig. 2). It is especially noteworthy that the RNA polymerases of R33, R39 and R47 showed a preference for the T7 DNA template, although they had the molecular weight of the T3 RNA polymerase.

Our main interpretations of the results are as follows. First, the higher molecular weight of the T7 RNA polymerase is probably not due to a single deletion which occurred in a T3 ancestor or a single insertion in a T7 ancestor. Rather, there seem to be at least two regions ('loops') of about equal size within gene 1 of T7, which have no homologous counterpart in gene 1 of T3. Otherwise, a recombination event leading to a gene 1 of a size intermediate between that of T3 and of T7 would be highly unlikely to occur. These loops (Fig. 3) comprise about 150–200 base pairs each, as judged by the physical length of gene 1, and may correspond to the region of nonhomology between T3 and T7, seen at about 0.15 genome length through electron microscopy of heteroduplex T3-T7 DNA (ref. 10). Second, gene 1 polypeptides whose primary structure over a large extent is that of the T3 enzyme but which carry the amino acid sequence encoded by one or both loops of T7 gene 1 are likely to fold into unstable tertiary structures; therefore the *in vitro* inactivity or temperature sensitivity of the recombinant enzymes. Third, the gene 1 segment which imparts the template specificity (either a preference for the DNA of T3 or T7) to the RNA polymerase is located at the 'right', that is the carboxyl end of gene 1. Thus, recombination events occurring distal to the loops of T7 gene 1 may lead to recombinant genes coding for RNA polymerases with a molecular weight characteristic of T3 but a template specificity characteristic of T7.

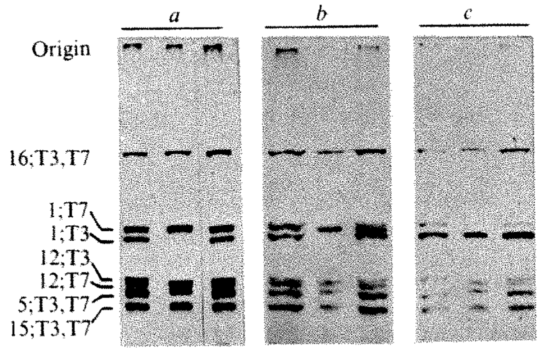

Fig. 2 Comparison of the molecular weight of recombinant RNA polymerases with that of T3 and T7 polymerase. Phage-directed proteins were continuously labelled by adding ^{35}S-methionine (40 μCi ml^{-1}, final concentration) together with the phage (multiplicity: 10 PFU per cell) to a sample (0.2 ml) of ultraviolet-irradiated *E. coli* B bacteria (3×10^8 cells ml^{-1}). After 15 min at 37° C the infected cells were collected by centrifugation and resuspended in 20μl of buffer (0.15 M Tris-Cl; 1.5 M 2-mercaptoethanol; 5% sodium dodecyl sulphate; 10% glycerin). After 2 h of incubation at 50° C, followed by 2 min at 100° C, the samples were placed on 8% polyacrylamide slab gels and subjected to electrophoresis for 5 h at 150 V. The dried gels were then autoradiographed for 2 d. Further details of the method are as described by Studier[12]. In this figure the results for three of the tested recombinants are given: *a* is an example of recombinant RNA polymerases with the molecular weight of the T7 polymerase (here: R34); *b* refers to R43, with a gene 1 product of intermediate molecular weight; and *c* represents the recombinant RNA polymerases with the molecular weight of the T3 enzyme (here: R47). For each recombinant tested, three parallel runs were made: at left is a reference frame which is a mixture of T3-coded and T7-coded proteins (the numbers at left refer to the corresponding genes[4,6]); the middle column is that of the recombinant proteins and at the right a mixture of T3, T7 and recombinant proteins was applied.

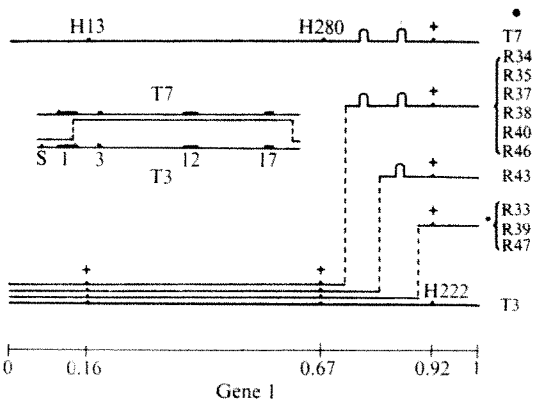

Fig. 3 Scheme of the presumptive genetic constitution of T3–T7 recombinants within gene 1. These recombinants were picked as am^+ plaques after plating the progeny of the cross T7*am*H13·*am*H280 × T3*am*H222·*am*H283 on the su^- host *E. coli* B. For the crossing procedure see ref. 3. The T7 parent carries two amber markers within gene 1 and the T3 parent is a gene 1–gene 3 double mutant. The position of the *am* markers within gene 1 was determined by estimating the molecular weights of the gene 1 amber peptides with the gel electrophoresis–autoradiography method[12]. The inset represents the length of the complete genomes of the parental phages[3,10] and the probable overall genetic constitution of the recombinants. The specified genes are those for which this constitution has been checked (S = *sam*). The presumed crossover at the right end of the genome is to satisfy the need for identical terminal redundancies[10] at the ends of recombinant phages; alternatively, such a crossover could have taken place to the left of the *sam* gene.

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DNA purified from naked intranuclear particles of human liver infected with hepatitis B virus

DANE *et al.*[1] observed a particle 42 nm in diameter with a double shell and an inner core resembling a viral nucleoid in the sera of some patients with hepatitis B antigenemia. They postulated that this particle was the complete hepatitis B virion. Almeida *et al.*[2] showed that an internal core particle, 27 nm in diameter, could be released by treating the 42 nm Dane particle with Tween-80 (polysorbate) and that this core particle contained an antigen different from hepatitis B antigen (HBAg). Almeida[3] suggested that these core particles were identical to naked particles, also 27 nm in diameter, observed by electron microscopy in the nuclei of hepatocytes of human livers infected with hepatitis B virus (HBV)[4,5]. Cores isolated from Dane particles in human sera[6] seem to be similar in morphology to the 27 nm naked particles visualised in homogenates of HBV-infected human livers[7]. We recently reported methods for isolation and purification of concentrated preparations of naked intranuclear particles of hepatocytes of HBV infected liver[8]. The ultraviolet absorption spectrum of these particles suspended in 0.05 M Tris-HCl, 40 mM KCl, 0.1% β-mercaptoethanol (BME), 10 mM $MgCl_2$, *p*H 7.5 buffer showed a peak at 264 nm and a shoulder at 280 nm indicating the presence of both nucleic acid and protein[8]. We now report the isolation of double-stranded DNA from these particles and describe the spectral properties of the isolated DNA.

The naked intranuclear particles of the hepatocytes of an HBV-infected human liver obtained at necropsy from a patient with chronic HBV infection were purified as previously described[8]. The purification procedure entailed the isolation of the liver nuclei, the subsequent release of the naked intranuclear particles from the nuclei by sonication, and the purification of the particles on CsCl density gradients[8]. The purified particles obtained from the CsCl gradients at a density of 1.30–1.31 gm ml^{-1} (ref. 8), and visualised by electron microscopy[8], were dialysed against 0.01 M NaCl, 0.01 M PO_4, 10^{-3} M EDTA, 1% BME, *p*H 7.2 buffer. The particle preparation was made up to a concentration of 1% of sodium dodecyl sulphate (SDS), allowed to stand at room temperature for 10 min, and then extracted four times at room temperature with distilled phenol previously equilibrated with buffer. The aqueous portion was dialysed exhaustively against two changes of 0.01 M NaCl, 0.01 M PO_4, 10^{-3} M EDTA, *p*H 7.2 buffer and then against four changes of 0.01 M NaCl, 0.01 M PO_4, *p*H 7.2 buffer at 5° C.

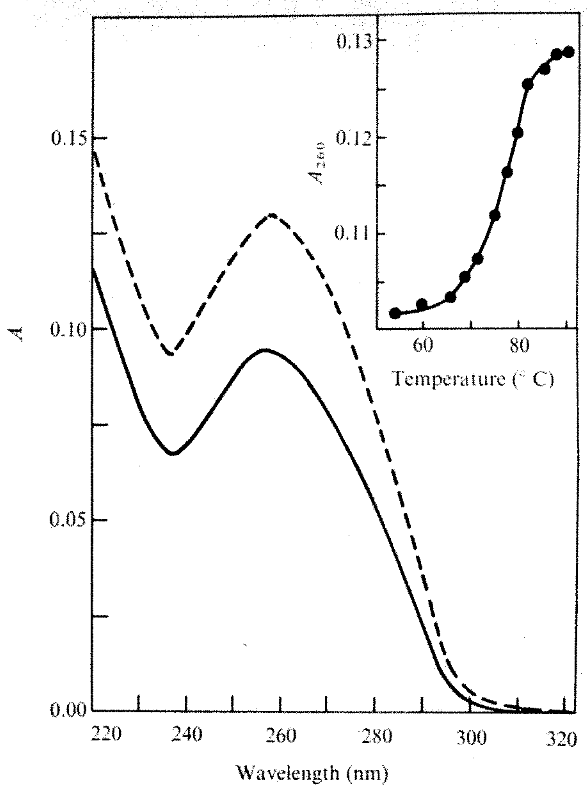

Fig. 1 Ultraviolet absorption spectra of DNA purified from naked intranuclear particles of hepatocytes of HBV-infected human liver. Solid line: native spectrum at 33.6° C; dashed line: denatured spectrum at 87° C. Insert shows the thermal denaturation profile at 260 nm. DNA was dissolved in 0.01 M NaCl, 0.01 M PO_4, *p*H 7.2 buffer. 100 μl microcuvettes of 1 cm pathlength contained 0.35 ml DNA solution to preclude meniscus effects on the absorbance values. The microcells were fitted with Teflon stoppers and sealed with RTV-60 compound. Temperature was controlled with a Laude K-2R circulation bath (Brinkmann Instruments) containing 50% ethylene glycol and measured by a platinum probe in a 3 ml sealed quartz cuvette filled with 1 ml buffer in the Beckman Acta V spectrophotometer. Temperature equilibrium was reached within 20 min at each point. The temperature was raised by 5° C increments until 50° C and then by 1–2° C until denaturation was complete. Absorbance values read by digital display at each temperature were corrected to 4° C for the expansion of water[13].

The ultraviolet absorption spectrum of the resulting dialysate suggested a solution of nucleic acid (Fig. 1). The nucleic acid was identified originally as double-stranded DNA by means of the following reactions: (1) a positive diphenylamine[9] but negative orcinol[10] reaction; (2) immediate increase in ultraviolet (220–290 nm) absorbance (+12% at 260 nm) when material of 0.1 absorbance at 260 nm was treated with pancreatic DNase (20 μg ml^{-1}; Sigma; double-stranded calf thymus and *Bacillus subtilis* DNAs gave comparable immediate increases in ultraviolet absorbance when treated with DNase under similar conditions, the immediate

Table 1 Properties of DNA purified from naked HBV particles determined by spectral analysis

	Hyperchromic spectra	Denatured spectra	
Mole fraction A–T	0.434±0.012 (s.d.)	0.374±0.023 (s.d.)	Average 0.404±0.036 (s.d.)
Concentration (mol nucleotide l^{-1})	1.35×10^{-5}	1.41×10^{-5}	DNA in native state (%), 95.7
Molar extinction at 260 nm (l mol^{-1} cm^{-1})	6,926	6,631	Average 6,779
Randomness (δ) parameter	0.012	0.124	

See text and legend to Fig. 1 for experimental details. Calculations were based upon data taken at 5 nm intervals between 220 and 290 nm for the hyperchromic spectra and at 240 nm, 250 nm, 260 nm, 270 nm and 280 nm for the denatured spectra using the three term analyses given by Hirschman and Felsenfeld[14]. A program written for a Wang 700B computer was used for the calculations.

increase in absorbance representing incomplete digestion of double-stranded DNA) and no change in absorbance when treated with pancreatic RNase (20 μg ml^{-1}, Sigma), (3) no reaction when neutralised formaldehyde was added to a concentration of 1 8% (ref 11), (4) a very slow increase in ultraviolet absorbance of the heat-denatured material treated with pancreatic DNase but no reaction when treated with pancreatic RNase, and (5) a slow increase (single-stranded RNA reacts immediately) in ultraviolet absorbance (~15% at 260 nm in 4 h) when the heat-denatured material was treated with neutralised formaldehyde (1 8%) Electron microscopic observation[12] of the material showed linear strands consistent with double-stranded DNA

First estimates from the absorbance at 260 nm indicated that the yield of double-stranded DNA from naked particles purified from 60 g of HBV infected liver was 15 μg It was considered that the properties of such modest amounts of DNA could well be studied by spectral analysis[13-15] adapted to microdeterminations Ultraviolet spectra were recorded with a Beckman Acta V spectrophotometer in the single beam mode using 100 μl microcuvettes and microapertures DNA of known base composition in 0 01 M NaCl, 0 01 M PO_4, *p*H 7 2 buffer also was analysed to determine the accuracy of the micromethods The microcells were fitted with Teflon stoppers and sealed with RTV silicone rubber compound (General Electric) for DNA melting experiments[13,14] Analysis of DNA of known base composition (*B subtilis* and calf thymus DNAs, 0 665 and 0 585 mole fraction A–T, respectively) adjusted to 0 1 absorbance at 260 nm at room temperature showed (1) accurate determination of the blank values of the microcuvettes was imperative, (2) correct assessment of the first temperature of complete denaturation[13] was critical, especially when deriving data from the denatured spectra, because the small changes in absorbance produced by depurination upon further heating[13] led to large errors in values of mole fraction A–T, (3) analyses of the denatured spectra gave lower values of A–T mole fraction, that showed greater scatter, than those derived from the hyperchromic spectra, (4) the low initial absorbance values required precise measurement of the temperature at each point because of the relatively greater contribution to the total absorbance values by the corrections for the expansion of water at each temperature, and (5) the microanalyses of DNA of known base composition were accurate to ±0 040 and ±0 055 mole fraction A–T using the hyperchromic and denatured spectra, respectively These values compare well with the macromethods[14] The native spectra were not used in these studies because of the greater inaccuracy of the derived values of mole fraction A–T[14] Only three-term analyses[14] were used to derive data from the spectra

The ultraviolet absorption spectrum of the purified native DNA showed a peak at 257 nm and a trough at 237 nm (Fig 1) When heated to 90° C the DNA gave a sharp rise in hyperchromism of 33% at 260 nm (Fig 1, insert), the melting temperature (T_m) in 0 01 M NaCl, 0 01 M PO_4, *p*H 7 2 buffer was 77° C, characteristic of DNA of 0 44 mol fraction A–T (S Z Hirschman, unpublished data)

The mol fraction A–T derived from the hyperchromic spectra (Fig 1) was 0 434±0 012 (standard deviation, s d) and from the denatured spectra 0 374 ± 0 023, the average value from both spectra was 0 404 ± 0 036 (Table 1) Analysis of the hyperchromic and denatured spectra yielded DNA concentrations of 1 35 × 10^{-5} and 1 41 × 10^{-5} mol nucleotide l^{-1}, respectively, indicating that 95 7% of the DNA was in native (double-stranded) state[14] (Table 1) The average molar extinction coefficient at 260 nm was 6,779 l mol^{-1} cm^{-1} (Table 1) The values of the 'randomness' (δ) parameters[13,14] were 0 012 and 0 124 for the hyperchromic and denatured spectra, respectively

Preliminary comparisons of the fraction of all A–T pairs (F_{AT}) against the fraction of all G–C (F_{GC}) pairs melted at each temperature[14,15] indicated that 60% of the DNA had a mol fraction A–T of 0 48 and 40% had a mol fraction A–T of 0 32 But because of the small changes in absorbance at each wavelength at each temperature throughout the melting these data will require substantiation by studies with much higher concentrations of DNA

Lower values of mol fraction A–T derived from the denatured spectra were found also with DNAs of known base composition (see above) The value of mol fraction A–T derived from the hyperchromic spectra agreed with the value obtained from the T_m at 260 nm The average extinction coefficient at 260 nm of 6,779 l mol^{-1} cm^{-1} compares to the extinction coefficient of 6,710 l mol^{-1} cm^{-1} previously reported for bacterial DNAs of 0 43 mol fraction A–T[13] The very low values of the 'randomness' (δ) parameter[13,14] suggested that the DNA did not contain abnormal bases

Thus, double-stranded DNA of unique base composition has been isolated from the putative HBV nucleoid, the naked intranuclear particle of hepatocytes of human liver infected with HBV Unfortunately, electron microscopic observation of the DNA showed the strands to be heterogeneous in size with a highest molecular weight of about 1 × 10^6 We assume that the DNA strands were broken either by liver nucleases or the sonication to release the particles from the nuclei of the hepatocytes during particle preparations[8] Various methods of particle preparation are under investigation to allow determination of the true molecular weight of the DNA

The small HBV nucleoid could accommodate a DNA of about 2 × 10^6 molecular weight Other small mammalian DNA-containing viruses, such as parvo[16] and adeno associated viruses (AAV)[17], contain single stranded DNA in the virion and may require a helper virus for replication The DNA of AAV may be packaged as separate plus and minus strands that can reassociate to a double-stranded form after extraction of the DNA[17] Whether HBV DNA is actually double stranded in the virion may still be open to some question But this question and the possibility that HBV may require a helper virus for replication can be subjected to experimental investigation now that concentrated naked particle preparations are available[8]

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Immunological destruction of herpes simplex virus I infected cells

THERE are clearly two levels at which the immune response may intervene to induce protection against viral infection Neutralising antibodies effectively limit spread of free virus in many situations, thus preventing reinfection In a number of viral diseases, however, it is often not possible to cure the infection by even high titres of neutralising antibodies[1,2] In such situations, recovery from infection may require the intervention of a cell-mediated immune response directed at the infected cell Selective impairment of the host's immunity, for example, by thymectomy or by antilymphocyte serum, markedly increased mortality of mice infected with ectromelia[3], and herpes simplex[4,5] Macrophage suppression has had a similar effect[6] In these cases recovery could be conferred by transfer of immune lymphoid cells (ref 7 and B R-Z, and A C Allison, unpublished) But the nature of the cells involved, and the mechanisms by which they act remain problematic Our experiments were undertaken to evaluate the contribution of various types of cells to immune reactivity to cells infected with herpes simplex virus I *in vitro*

Adult C57Bl/6J mice were infected intraperitoneally with 10^6 plaque-forming units (PFU) of the HFEM strain of herpes simplex virus I, (HSV), grown in chick embryo fibroblasts, and immune lymphoid cells were collected at various times after immunisation The ability of these cells to destroy HSV infected Chang human liver cells, was assessed by measuring the release of ^{51}Cr from labelled HSV-infected Chang cells 16 h after treatment with various immune or normal lymphoid cells In this system, Chang cells in suspension were infected at an input multiplicity of 10 Twenty hours after infection, when lymphoid cell preparations were added, 80% of the cells exhibited viral specific membrane fluorescence Infected cells were labelled for 45 min at 37° C with 100 μCi ^{51}CrO$_4$ (Amersham) washed three times, and 5 × 10^4 cells were distributed to plastic test tubes Lymphoid cells were added at a ratio of 40 1 and the cells were cultured in Eagle's medium containing 6% foetal calf serum Spontaneous ^{51}Cr leakage was approximately 15–20%

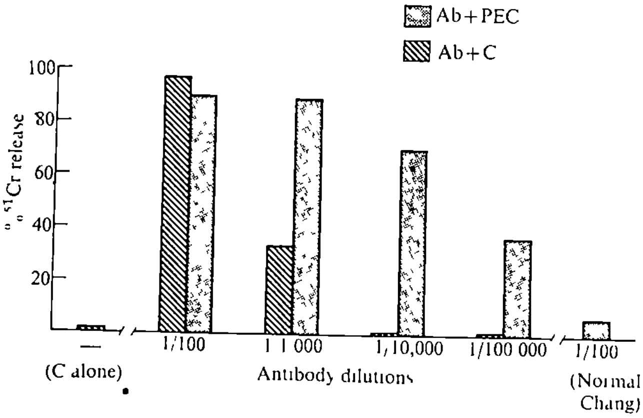

Fig 1 Comparative efficiency of cytolysis of HSV-infected Chang cells by Ab-dependent cell-mediated lysis and complement (C)- mediated lysis Target cells were treated with dilutions of rabbit anti-HSV serum before the addition of effector cells or complement The neutralisation titre of the anti-HSV serum was 1 640 Complement was used at a 1 12 dilution of fresh guinea pig serum The ratio of PEC to target cells was 40 1

Table 1 Cytolysis of HSV-infected Chang cells by immune or nonimmune mouse lymphoid cells

Target cells	Effector cells	% Specific ^{51}Cr release
HSV-infected Chang	Nonimmune spleen cells	0
	Immune spleen cells	4 5
	Nonimmune spleen cells + anti-HSV mouse serum (1/100)	4 5
	Immune peritoneal exudate cells	46 0
	Nonimmune peritoneal exudate cells + normal mouse serum (1/100)	0
	Immune spleen cells + nonimmune peritoneal exudate cells (20 1)	30 0
	Nonimmune peritoneal exudate cells + anti-HSV mouse serum (1/100)	90 0
	Anti-HSV mouse serum (1/100)	2 5
Noninfected Chang	Nonimmune peritoneal exudate cells + anti-HSV mouse serum (1/100)	7 0

Mouse anti-HSV serum was added to 5 × 10^4 target cells in plastic test tubes (Falcon No 2054) Effector cells were then added at a ratio of 40 1 in a final volume of 1 0 ml Samples were prepared in triplicate Percentage lysis was computed as
(Mean ^{51}Cr release in test group—mean spontaneous ^{51}Cr release)/(Mean ^{51}Cr release after freeze-thawing—mean spontaneous ^{51}Cr release) × 100

Repeated attempts to demonstrate the presence of cytolytic lymphocytes in immune spleens were unsuccessful, even in the presence of anti-HSV antibodies (Table 1) In contrast, peritoneal exudate cells (PEC) from immune donors 6 d after infection were markedly cytotoxic for HSV-infected Chang cells When peritoneal exudate cells from normal mice were admixed with immune spleen cells (at a ratio of 1 20), a significant degree of cytotoxicity was observed, suggesting that the cytotoxicity might be mediated by nonimmune effector cells This was confirmed by the demonstration that nonimmune peritoneal exudate cells in the presence of heat-inactivated anti-HSV rabbit or murine serum had an even greater degree of cytotoxicity than immune peritoneal cells Immune sera from mouse, rabbit or man were all effective, as demonstrated in Table 2 and both mouse exudate or human peripheral monnuclear cells could serve as effector cells

The efficiency of cytolysis of HSV-infected cells by antibody-dependent cell-mediated lysis and complement-mediated lysis is compared in Fig 1 At high concentrations of rabbit anti-HSV antibody, high degrees of lysis of HSV infected cells were observed in both systems The efficiency of complement-mediated cytolysis however, diminished markedly upon dilution of antibody, whereas the cytotoxicity mediated by

Table 2 Cytolysis of HSV-infected Chang cells by both mouse peritoneal exudate (PEC) or human peripheral blood mononuclear cells (PBL) in the presence of either rabbit or human immune sera

Effector cells	Serum	% Specific ^{51}Cr release
PBL	Nonimmune rabbit 1/100	25
PBL	Rabbit anti-HSV 1/100	83
PBL	Nonimmune human 1/100	31
PBL	Human anti-HSV 1/100	81
PEC	Nonimmune rabbit 1/100	10
PEC	Rabbit anti-HSV 1/100	89
PEC	Nonimmune human 1/100	15
PEC	Human anti-HSV 1/100	81

Nonimmune human serum and peripheral blood were taken from donors with no known previous HSV infection Immune human serum was taken from a patient with recurrent HSV infections Human peripheral blood mononuclear cells were prepared by Ficoll-Hypaque gradient centrifugation[16] In all cases the lymphocyte target cell ratio was 40 1 nonimmune peritoneal exudate cells remained high even in the presence of a 1/10,000 antibody dilution A similarly pronounced effect was observed in the case of murine anti-HSV antibodies obtained early (6 d) after immunisation, in which 52% chromium release was obtained in the presence of normal peritoneal exudate cells with but 3 5% in the presence of guinea pig complement

These observations confirm the prediction of Allison[1] that antibody-dependent cell-mediated cytotoxicity would be operative in viral immunity, and extend the fundamental studies of Perlmann[8], MacLennon[9] and Evans and Alexander[10] to the case of virus-infected cells Although the precise nature of the effector cell(s) in these systems has not yet been fully elucidated, it clearly does not share characteristics with thymus-derived lymphocytes[1,9] Whether it belongs to a subpopulation of B lymphocytes macrophages or is a novel cell type remains unclear, but the effector cells in the mouse have been found to be surface-adherent, poorly phagocytic and dependent on an Fc receptor Here we confirm that removal of adherent cells from the peritoneal exudate population by culture for 2 h in 60 mm plastic Petri dishes results in a 90% diminution in cytotoxic activity of the cells

In their studies on the part played by antibodies and cell-mediated reactions on spread of HSV infection *in vitro*, Lodmell *et al*[11] noted that spread of infection had occurred as early as 2 h before the detection of surface HSV antigen by complement-mediated cytotoxicity, and suggested that consequently antibodies were not likely to be useful in limiting the spread of infection Our studies indicate that antibody-dependent cell-mediated cytolysis of HSV infected cells provides a mechanism orders of magnitude more efficient than complement-mediated cytolysis in terms of antibody required While extrapolation of the *in vitro* results to the *in vivo* situation would be premature, the data suggest that a collaboration of antibody and the appropriate effector cells could provide a significant degree of restriction of infection In addition, other cell-mediated mechanisms, such as T-cell dependent cytotoxicity[12,13] and possibly generation of mediators as a consequence of the inflammatory response[14,15] may be involved

Note added in proof Similar results have recently been obtained independently by Shore, S L, Nahmias, A J, Starr, S E, Wood, T A, and McFarlin, D E, *Nature* (in the press)

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Pronounced antiviral activity of human interferon on bovine and porcine cells

INTERFERONS prepared from cells of one species may exhibit some activity on cells of others although the titre on heterologous cells is usually considerably lower than that for homologous cells[1] The one exception to date has been the finding that human fibroblast interferon was far more active on heterologous rabbit cells than on human cells[2,3] We report here that human leukocyte and fibroblast interferon preparations exert a pronounced antiviral activity on bovine and porcine cells, and the activity of the human leukocyte interferon is far greater on heterologous bovine cells than on homologous human or monkey cells

The origins of the interferon preparations and the cells used in their assay are presented in Table 1 Several interferon preparations were prepared in our laboratory and standard techniques were used to eliminate residual inducing virus All cells were cultivated in Eagle's medium with 10% calf or foetal calf serum Dilutions of interferon preparations were incubated with cell cultures for 18 h before the test virus was added Two methods of assay were used First, inhibition of the cytopathic effect (CPE) of 100 $TCID_{50}$ of vesicular stomatitis virus (VSV) or encephalomyocarditis (EMC) virus in monolayer cultures of cells in plastic Microtest II Trays (Falcon)[7] Titres are expressed as the highest two-fold dilution giving approximately 50–75% protection of the cell sheet at a time when virus-infected control cultures showed complete cell sheet destruction Second, inhibition of virus yield determined 12 or 18 h after addition of VSV or EMC virus at a multiplicity of infection of 1 Titres are expressed as obtained in our laboratory (One of our units is approximately the equivalent of 1 5–2 human leukocyte MRC reference interferon 69/19 units)

The antiviral activity of human interferon preparations

Table 1 Interferon preparations

Tissue origin	Interferon inducer	Supplied by
Human leukocyte suspensions	Sendai virus	Dr K Cantell Dr H Strander[4,5]
Human leukocyte suspensions	Newcastle disease virus (NDV)	Dr T Kishida[6]
Human leukocyte suspensions	Sendai virus	Medical Research Council Research standard B 69/19
Human Burkitt cells 'Namalwa line'	NDV	Our laboratory
Human embryonic fibroblasts	Poly(I) poly(C)	Dr P Came
Human embryonic fibroblasts	NDV	Our laboratory
Mouse L cells	NDV	Our laboratory
Rabbit RK_{13} cells	NDV	Our laboratory
Bovine embryonic skin	NDV	Our laboratory
Porcine (adult) kidney	NDV	Our laboratory

Cells used in assay of interferon

Species	Cell	Passaged used	Supplied by
Human	Fibroblast WI-38	20 to 28	Merieux Laboratories
Monkey	BSC (kidney)	Established cell line	Our laboratory
Cow	Embryonic skin	1 to 15	Our laboratory
Cow	Primary kidney	1 to 6	Merieux Laboratories
Pig	Primary kidney	1 to 6	Merieux Laboratories
Pig	PK_{106} (kidney)	Established cell line	Dr A Parodi and Dr R Scherrer
Pig	PK (Kasza)	Established cell line	Flow Laboratories

Table 2 Antiviral activity of human interferon preparations on human, monkey, bovine and porcine cells

		Titre* of interferon on cells challenged with VSV						
		Human	Monkey	Bovine		Porcine		
Origin of interferon preparation	Species cell type	WI-38 fibroblast	BSC	Embryonic skin	Adult primary kidney	Adult primary kidney	PK_{106}	$PK_{(Ka8/a)}$
Leukocyte suspensions	Cantell	640,000	640,000	25,000,000	51,000,000	1,200,000	640,000	—
		640,000	1,200,000	25,000,000	12,000,000	—	640,000	640,000
	Kishida	4,000	4,000	32,000	120,000	25,000	3,200	—
		4,000	8,000	120,000	120,000	—	12,000	6,400
	MRC	3,200	3,200	51,200	—	—	—	—
	reference	—	3,000	64,000	—	—	—	—
	69/19	2,400	2,400	—	—	3,200	2,400	1,600
Burkitt cell line 'Namalwa'	Our laboratory	—	1,280	10,240	—	—	—	—
Embryonic fibroblast	Came	6,400	12,800	3,200	—	—	—	—
		—	19,200	—	—	400	1,200	600
		—	12,800	6,400	—	—	—	—
	Our laboratory	—	320	160	—	—	—	—
Heterologous Mouse L cells (titre 1 1,600 on mouse L cells)		—	—	<20	—	—	<20	—
Rabbit RK_{13} cells (titre 1 3,200 on RK_{13} cells)		—	—	<20	—	—	<20	—
Bovine embryonic skin		40	—	6,400	—	—	—	—
Porcine kidney		<10	—	—	—	1 160	—	—

* Titre is given as the reciprocal All titres on the same line represent the values obtained in simultaneous assays on a given day
—, Not tested

on human, monkey, bovine and porcine cells using VSV as the challenge virus is presented in Table 2 and the salient points may be summarised as follows

Three different human interferon preparations derived from leukocyte suspensions (including the MRC standard reference interferon 69/19, a partially purified interferon (Kishida) and a potent preparation (Cantell) used in the treatment of patients[5]) all protect bovine cells to a far greater extent than they protect either human fibroblasts or BSC cells For example, the Cantell preparation had a titre of 1 64×10^4 on human fibroblasts or monkey kidney cells and a titre of 1 25×10^6 on bovine embryonic cells

Human leukocyte interferon preparations protect porcine kidney cells to the same degree as human fibroblasts or BSC cells

Interferon prepared from a continuous Burkitt cell line behaves as an interferon derived from normal leukocyte suspensions and protects bovine cells to a greater extent than BSC cells

Human embryonic fibroblast interferon (induced either by poly(I), poly(C) or NDV—Table 1) crosses on bovine and porcine cells In contrast to leukocyte interferon, however, the antiviral titre of fibroblast interferon was slightly less on bovine cells and considerably less on porcine cells than on human or monkey cells

Although human interferon preparations exhibited antiviral activity on bovine and porcine cells, these cells are not protected by mouse or rabbit cell culture interferon preparations

Bovine embryonic skin interferon exhibited only slight antiviral activity, and porcine kidney interferon exhibited no detectable activity on human fibroblasts

The pronounced antiviral activity of human leukocyte interferon on bovine and porcine cells and the less pronounced activity of human fibroblast interferon on these cells were also observed when EMC virus was used as a challenge virus instead of VSV Likewise, the pronounced antiviral activity of the human leukocyte reference interferon on bovine cells was observed when assayed by inhibition of VSV or EMC virus yield rather than by inhibition of CPE

A number of tests were undertaken using the MRC reference interferon 69/19 to determine the characteristics of the factor responsible for protection of bovine and porcine cells As can be seen in Table 3, this factor fulfilled the usual criteria for acceptance of a viral inhibitor as an interferon[1] Furthermore, a rabbit antihuman leukocyte interferon serum neutralised (to a titre of 1 800) 4 units of the human leukocyte reference interferon 69/19 as determined in simultaneous assays on monkey, bovine and porcine cells

Table 3 Characteristics of human leukocyte reference interferon 69/19 as tested on monkey (BSC) bovine embryo (BE) and porcine kidney (PK) cells

Test	Activity on cells BSC	BE	PK_{106}
Washing (× 2) of cell sheet before the addition of challenge virus	++	++	++
Requires several hours incubation on cells for establishment of antiviral activity	++	++	++
Pretreatment of cells for 1 h with 5 μg actinomycin D	±	0	0
*p*H 2 for 24 h	++	++	++
Dialysis for 24 h	++	++	++
Supernate after centrifugation 100,000*g* 1 h	++	++	++
Heating at 60° C for 1 h	+	+	+
Trypsin 500 μg for 1 h 37° C	0	0	0
DNAase or RNAase 100 μg 1 h 37° C	++	++	++

VSV used as a challenge virus in these tests ++, Activity undiminished by treatment, +, approximately 50% loss of activity, ±, approximately 50–90% loss of activity, 0 = > 95% loss of activity

The finding that human leukocyte interferon exhibited a far greater activity on bovine and porcine cells than human fibroblast interferon of comparable titre is in accord with previous work suggesting that these two interferons differ in their biological[2,3] as well as in their physicochemical properties (V Edy, A Billiau, M Joniau, and P De Somer, unpublished and W E Stewart II, P De Somer and E De Clercq, unpublished)

The finding that human leukocyte interferon has such a high titre on bovine cells (that is, Cantell's human leukocyte interferon had a titre of 1 25×10^6 on bovine cells) may be of value in veterinary medicine Thus, it may prove simpler to prepare human leukocyte interferon (perhaps from a leukocyte cell line) than to prepare bovine interferon preparations of comparable titre Furthermore, since bovine cells are highly sensitive to human leukocyte interferon, they may prove useful for the detection of small amounts of this interferon

It has been suggested[8] that cells contain specific interferon receptors that may be responsible for the usual species specificity of interferon If such receptors exist, our results pose the question whether bovine and porcine cells have receptors for both homologous and human interferon or whether these cells share a common interferon receptor

At least three hypotheses may be suggested to explain why human leukocyte interferon exerts a greater activity on bovine cells than on homologous cells First, fewer molecules of human leukocyte interferon may be needed to induce antiviral activity in bovine cells than in human cells Second, there are more interferon receptors on bovine cells than on human cells Therefore, at limiting dilutions of interferon there is a greater chance of inducing antiviral activity in bovine cells than in human cells Third, an inhibitor of human leukocyte interferon activity is present in human cells but not in bovine cells

The explanation of such high activity of interferon on unrelated heterologous cells should prove relevant to the understanding of the nature of the interaction of interferon with cells and the mechanism of the usually observed limited activity of interferon on heterologous cells

We thank Dr Cantell for supplying rabbit anti-human leukocyte interferon serum, Drs Cantell, Strander, Kishida and Came for human leukocyte and fibroblast interferons, and Dr S G Anderson who made available to us the MRC reference interferon We also thank Drs Parodi and Scherrer for giving us the porcine kidney cell line and Dr Strander for the Burkitt cell line 'Namalwa'

Note added in proof Since submission of this article, we have shown that the human leukocyte reference interferon 69/19 and the Cantell human leukocyte interferon also exhibit a far greater antiviral activity on two other bovine cells, embryonic lung (passage six) and embryonic kidney (passage three to five) than on homologous human fibroblasts or monkey kidney cells

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Reactivation of immunocompetence in spleen cells of aged mice

REACTIVITY in both humoral and cellular immune reactions has been shown to decline with age[1–4], but the reasons have not yet been critically established It could be attributed, at least in part, to thymus involution, since the most prominent reduction in reactivity is shown in thymus-dependent responses[5] The question is, therefore, whether decreasing immune reactivity results from a decrease in the stem cell pool, or whether it is due to a failure in one of the processes of cell maturation which are affected by the thymus

This study was designed to distinguish between these two possibilities We used the graft-*versus*-host (GvH) response as the experimental model, since a decrease in the potential to elicit this response during ageing has been reported[6] Thus, spleen cells originating in old mice, when injected into allogeneic newborn recipients, produced splenomegaly reactions at a lower level than cells of young mice To ascertain that the failure to cause splenomegaly does not result from different homing patterns of the cells introduced to the newborn test animal, we used the *in vitro* technique for the GvH response[7] We first attempted to establish at which age the reduction in activity is most apparent in the mice used in this study Thus, (C3H/eb×C57Bl)F_1 mice were tested at four different ages 2–3, 12–15, 23–26 and 32–33 months 5×10^5 spleen cells of each of these groups were added to explants of newborn BALB/c spleens The relative enlargement of these newborn spleen fragments as compared with fragments cultured with syngeneic spleen cells was recorded after 4 d, as previously described[7] As shown in Fig 1, a decrease in the ability of spleen cells to elicit a splenomegaly reaction *in vitro* is manifested with advancing age The 2–3 month old mice (Group *a*) elicited the highest incidence of response High incidence of response was also

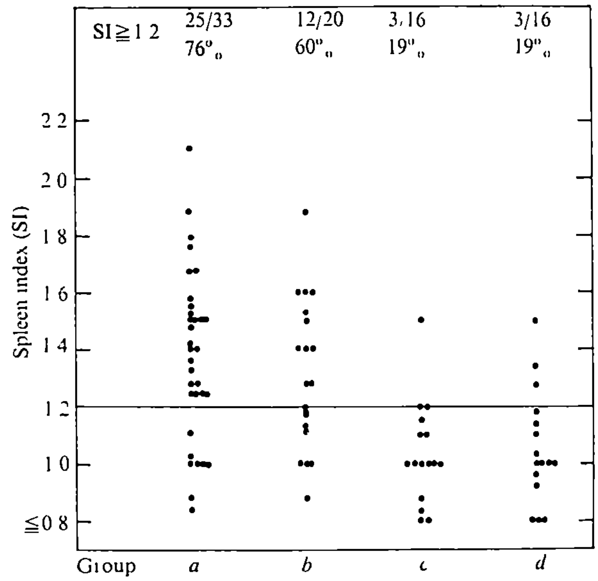

Fig 1 *In vitro* GvH response by spleen cells of aged mice Index of newborn BALB/c spleens following incubation with 5×10^5 spleen cells of (C3H/eb×C57Bl)F_1 mice at different ages Each point represents the results of one individual culture Group *a*, 3 month old mice, *b*, 12–15 month old mice, *c*, 23–26 month old mice, *d*, 32–33 month old mice

obtained in the group of 12–14 month old mice (Group *b*). Cells of the 23–26 (Group *c*) however, and those of the 32–33 month group (Group *d*) manifested a limited reactivity. These *in vitro* results eliminate the possibility that different homing mechanisms account for the failure to produce a response, since the cells were applied directly to the target organ, the newborn spleen. So a reduction in the immune potential of the cells from the aged mice is suggested.

This was further substantiated by increasing the cell doses used to elicit the response. The activity of 10^6 spleen cells from 32–33 month-old mice was tested in comparison with 5×10^6 cells. As seen in Fig 2, group *a*, the larger dose of cells did lead to a higher incidence of response, 75% as compared to 43%, similar to a higher reactivity obtained upon increase of dose of cells from young mice (Group *b*) (94% as compared to 68%, respectively). Yet, even at that higher dose of cells, the response obtained was relatively low compared to that of the young. These results could be attributed to a decrease in number of progenitor cells, or to a failure of maturation of progenitors to immune reactive cells as a result of thymus involution. To test this, we planned to apply thymus extract (THF)[8], since it has been shown to cause cells to become reactive in a GvH response[9]. The *in vitro* GvH experiment was thus repeated, employing spleen cells of 23–26 months, at a dose of 5×10^5 cells, and THF was added to the culture medium, at a concentration of 2% for the entire period of culture[10]. Cultures without THF were set up in parallel. Similarly, cells of 2–3 months old mice were tested with and without THF. As shown in Fig 3, THF enhanced the response elicited by cells from aged donors (Group *a*), whereas it did not lead to any change in reactivity of the cells from young animals (Group *b*).

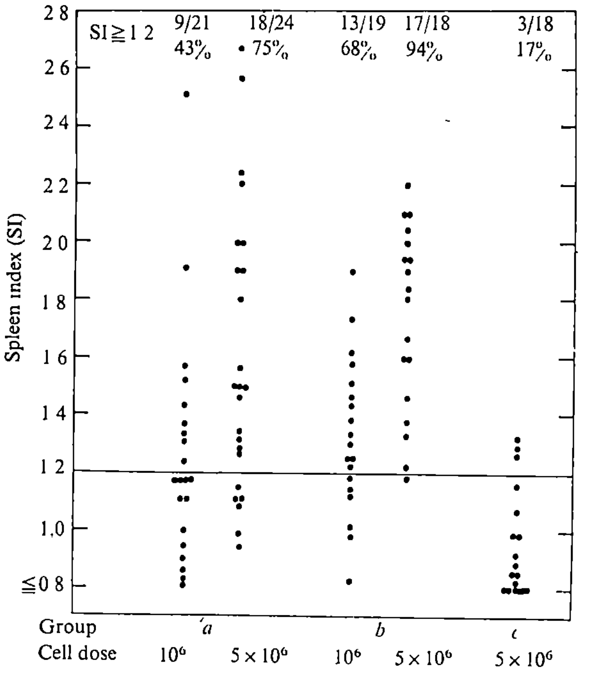

Fig. 2 *In vitro* GvH response by spleen cells of aged mice. Index of newborn BALB/c spleens following incubation with 10^6 or 5×10^6 spleen cells from mice at different ages. Each point represents the results of one individual culture. Group *a*, 32–33 month old (C3H/eb×C57Bl)F$_1$ mice, *b*, 3 month old (C3H/eb×C57Bl)F$_1$ mice, *c*, 3 month old BALB/c mice.

These results cannot be explained by a deficit in progenitor cells, since no cells were supplied to the system from any external source. We suggest that the decline in the ability to produce a GvH response during ageing stems from inadequate activity of thymus hormone, resulting in a failure of maturation.

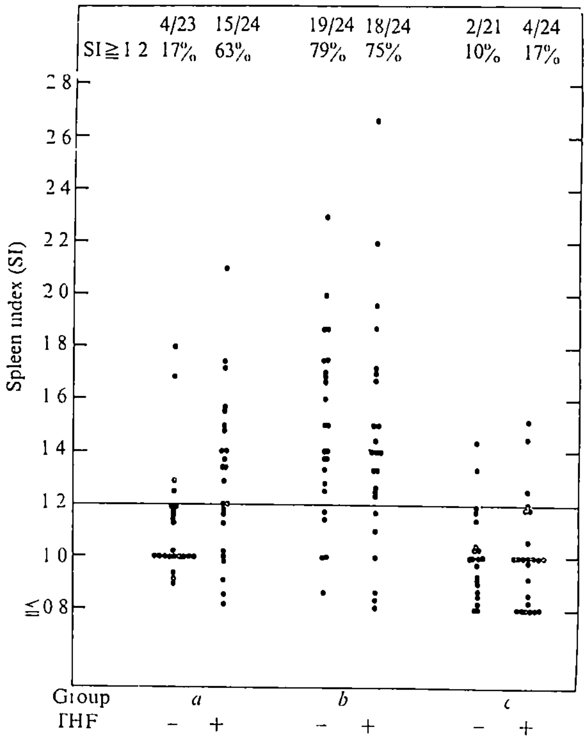

Fig 3 *In vitro* effect of THF on the ability of spleen cells of aged mice to elecit a GvH response. Index of newborn BALB/c spleens following incubation with 5×10^5 spleen cells from mice at different ages. Each point represents the results of one individual culture. Group *a*, 23–26 month old (C3H/eb×C57Bl)F$_1$ mice, *b*, 3 month old (C3H/eb×C57Bl)F$_1$ mice, *c*, 3 month old BALB/c mice.

In earlier studies[11,12] grafting of young thymus tissue into aged mice had no effect on the response. This may be due to the hormonal activity of the grafts being depressed by central control mechanisms[13] of the aged recipients.

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Immunological surveillance against altered self components by sensitised T lymphocytes in lymphocytic choriomeningitis

THE cytotoxic activity[1,2] of immune thymus-derived lymphocytes (T cells) for ^{51}Cr-labelled fibroblasts, or macrophages infected with lymphocytic choriomeningitis (LCM) virus is restricted by the H-2 gene complex[3 4] Specific lysis of LCM-infected monolayer cultures occurs only when targets and overlaying, sensitised T cells share at least one set of H-2 antigenic specificities

Operationally, this restriction may reflect one of two quite distinct mechanisms[1,2] First, H-2 compatibility is essential for sufficiently close association[3] between lymphocyte and target cell for lysis to occur This intimacy model implies either that the H-2 gene complex specifies a product, or products, involved in self-recognition, or that there is some mutual interaction between H-2 antigens Such a process would be additional to recognition of viral antigen by the T cell receptor The alternative possibility is that infection with LCM virus modifies self components in a way recognised only within a H-2 compatible system Altered self may be thought of as changes in H-2 antigens (or in structures coded for in the H-2 region) induced by the process of virus synthesis, or as some complex of viral and H-2 antigens

Lymphocytes from F_1 immune mice lyse virus-infected targets of parent H-2 type as effectively as do syngeneic T cells[1] If the intimacy model is correct, therefore, F_1 mice need possess only one clone of sensitised T cells, recognising viral antigen by a specific receptor and like H-2 antigen, of either parent type, in some non-immunological way (Fig 1) The altered self hypothesis, however, requires the presence of at least two clones of T cells, each reactive to modified H-2 of one parent type

These possibilities have been examined using the following experimental system When LCM-immune T cells are injected into immunosuppressed[5], virus-infected recipients they home equally well to lymphoid tissue of syngeneic or allogeneic hosts, but continue to multiply only in the syngeneic system[6] Further replication is apparently not triggered by free virus, but is dependent on thymus-derived lymphocytes being exposed to histocompatible, virus-infected target cells

Table 1 Cytotoxic activity of donor T cells in LCM-infected irradiated recipients*

Donor cells*	Recipient strain	H-2 type	% ^{51}Cr release† from L cells (H-2^{k}) Infected	Normal
Experiment 1				
Immune	CBA/H × BALB/c	k/d	51 2 ± 0 5	12 5 ± 1 9
	C57Bl × BALB/c	b/d	17 3 ± 0 1	13 9 ± 1 3
	BALB/c	d	16 1 ± 1 8	13 0 ± 1 3
Normal	BALB/c	d	14 4 ± 1 9	11 7 ± 1 1
Experiment 2				
Immune	CBA/H	k	83 7 ± 5 4	19 0 ± 1 4
	C57Bl	b	23 3 ± 1 3	19 1 ± 1 0
Normal	C57Bl	b	20 3 ± 1 2	18 5 ± 1 7

*Donor CBA/H × C57Bl F_1 were injected intracerebrally with 300 LD50 of WE3 LCM virus and killed when clinically affected 7 d later Recipients were irradiated (850 r) 24 h before i v injection of 10^6 LD50 of WE3 LCM virus, and inoculated i v with 5 0 × 10^7 spleen and lymph node cells 6 h later

†% ^{51}Cr release above normal values is a measure of activity of H-2 compatible sensitised T cells[1-4] Spleen cells from LCM-immune mice caused % ^{51}Cr release of 46 6± 0 9% from infected L cells and 15 2± 1 3% from normal L cells (experiment 1)

Table 2 Evidence that sensitised T cells are of donor origin

Donor	Recipient	Treatment	% ^{51}Cr release from L cells Infected	Normal
CBA/H × C57Bl F_1	AKR	Anti-θ+C	22 3 ± 1 2	21 4 ± 2 5
H-2$^{k/b}$, θC3H	H-2^{k}, θAKR	N ascitic+C	89 1 ± 1 8	23 3 ± 1 2

The protocol is the same as in Table 1, except that a proportion of lymphoid cells were treated[3] with AKR anti-θ (C3H) ascitic fluid and rabbit complement or normal AKR ascitic fluid and complement

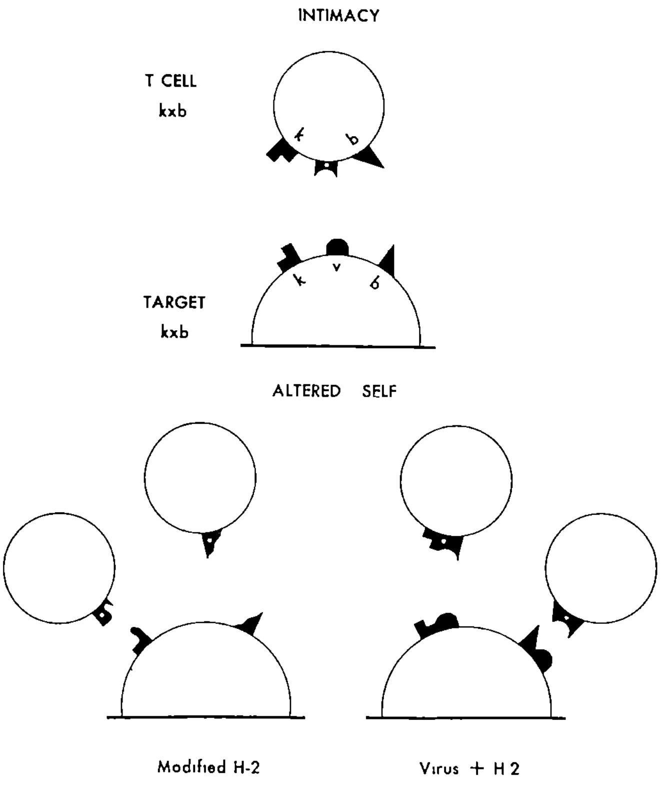

Fig 1 Capacity of sensitised F_1 (H-2$^{k/b}$) T cells to interact only with histocompatible virus-infected target cells may be considered to reflect any one of the models shown The intimacy concept proposes a single immunologicslly specific T cell receptor for viral (v) antigen, additional to a requirement for physiological interaction coded for by the H-2 gene complex (mutuality between either H-2^{k} or H-2^{b}) The two models proposed for altered self postulate that, in each case, there are at least two T cell populations with receptors of different immunological specificities recognising modified H-2, or virus +H-2 of either parent type

Continued proliferation of F_1 (H-2$^{k/b}$) immune spleen cells with capacity to lyse H-2^{k} LCM-infected monolayers occurs only in recipients with H-2^{k} antigenic specificities, and not in those possessing H-2^{d} (Table 1) These sensitised T cells are of donor origin, as lymphocytes recovered from H-2 compatible AKR recipients adoptively immunised with CBA/H × C57Bl spleen cells were shown to bear θ C3H (Table 2) The intimacy hypothesis, therefore, is probably not correct, as the postulated single clone of F_1 lymphocytes (Fig 1) should continue to proliferate equally well in recipients of either parent strain The results cannot, however, exclude this model totally, the reservation being that there may be alleleic exclusion in the F_1 of this hypothetical self-recognition product The simplest explanation of our results is, however, that there are sensitised T cells of at least two specificities in LCM-infected $H\text{-}2^{k/b}$ mice, each recognising a complex of virus + H-2 (or modified H-2) of one parent type Recirculating T cells may function essentially to survey the integtity of transplantation antigens, or structures coded for by the H-2 gene complex Recognition of cell surface changes due to virus infection, chemical modification[7] or genetic difference (alloantigens) may then be accommodated within the same model

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Immunodeficiency and runting syndrome in rats from congenital pyridoxine deficiency

CONGENITAL pyridoxine deficiency is teratogenic in the rat, producing digital defects, cleft palate, omphalocoele, micrognathia, exencephaly and hypoplasia of the spleen and thymus[1] Surviving offspring may develop convulsions[2-4], presumably because of a delay in the development of the γ-aminobutyrate shunt in the brain[5] Congenital pyridoxine deficiency also causes cleft palate in the mouse[6] I have now found that congenital pyridoxine deficiency has an adverse effect on the immunological function of surviving pups

I used six dietary groups one with congenital pyridoxine deficiency (group *a*) and five control groups (*b*–*f*) Virgin female Sprague–Dawley rats were placed with males in the evening The morning after mating, when copulatory plugs or sperm could be found, was counted as day 0 To obtain group *a*, we gave pregnant rats pyridoxine-deficient diet (Nutritional Biochemicals) with 0 1 mg ml^{-1} 4-deoxypyridoxine (4-D), an antagonist of pyridoxine in their drinking water from day 4, 5 or 6 to day 21 of gestation, when they were returned to a regular diet and tap water All these rats developed dermatitis and other clinical signs of pyridoxine deficiency which disappeared within a few days after resumption of the regular diet Convulsions were not observed among the newborn offspring, possibly because the dams were given the regular diet before delivery To obtain group *b*, I gave pregnant rats pyridoxine-deficient diet with antagonist in the water and supplemental pyridoxine (1 0 mg ml^{-1}) in the drinking water from day 5 to day 21 of gestation Group *c* received the regular diet and tap water throughout gestation Group *d* was given 8 g of the regular diet each day, which constituted a dietary restriction severe enough to limit maternal and foetal growth without causing anomalies in Sprague–Dawley rats[7] Group *e* was given 8 g per day of pyridoxine-deficient diet with antagonist and excess pyridoxine in the drinking water because of the possibility that a restricted intake of the deficient diet might lead to some deficiency other than pyridoxine Group *f* was given equal molar concentrations of antagonist and pyridoxine in the drinking water

Mean weights for the litters for the first 4 weeks of life are in Table 1 Rats in *d* and *e* were small at birth while those in all control groups grew rapidly for the first 4 weeks No control pup developed runting Rats in litter 1 of group *a* grew slowly A male rat in litter 1 and a male rat and three females in litter 2 developed a runting syndrome

Rats were weaned at 28 d of age, individually identified with ear tags and separated in cages according to sex Rats in group *a* which did not develop a runting syndrome grew vigorously and all rats in the five control groups grew vigorously Weights are shown in Table 2 for the second month of life for male rats in group *a* and group *c* Two females in litter 2 with the runting syndrome died between 32 and 39 d of age A third female runt in that litter was killed at 60 d of age when its weight was 43 g

As shown in the Tables, there was considerable variation from litter to litter with respect to the frequency of runting and the rate of growth In some litters all rats grew normally and there was no runting

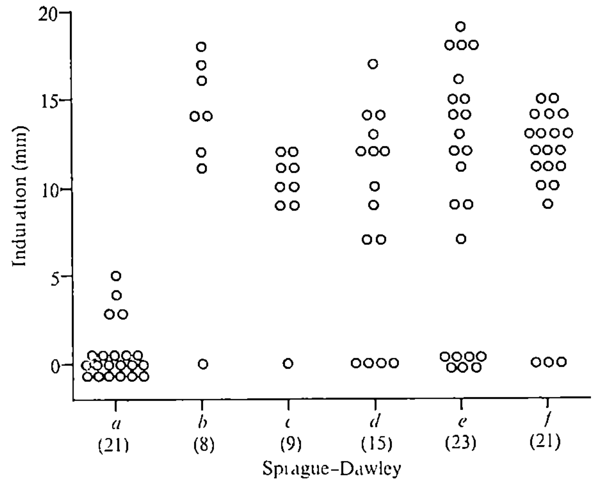

Fig 1 Results of tuberculin test at 8 weeks of age

I next bred 12 female Sprague–Dawley rats and gave them the diet for group *b* from day 5 to day 21 of gestation Two rats were not pregnant (17%) The average maternal weight gain was 155 g The 10 pregnant rats gave birth to 114 live and one dead offspring The average birth weight was 7 0 g No anomalies were recognised among the offspring, and none of them developed a runting syndrome All survived to 6 weeks of age when immunisations were begun

Thirty-eight rats were bred and given dietary treatment *a* from day 5 to day 21 of gestation Five rats were not pregnant (13%) Two pregnant rats died in late gestation, and two died on the day of delivery The average maternal weight gain was 20 g Of the 429 pups born, 207 died or were eaten on the first day Average birth weight was 4 8 g Ninety-four other pups died before 6 weeks of age, leaving 128 alive (30%) Eight of the survivors had developed a runting syndrome by 6 weeks of age Several rats with a runting syndrome were killed and examined histologically

Table 1 Average weights in the first month of life of rats treated with congenital pyridoxine deficiency (group *a*) or with five control diets (groups *b–f*)

Age (d)	Group *a* Litter 1		Group *a* Litter 2		Group *a* Litter 3		Group *a* Litter 4		Group *b* (2 Litters)		Group *c* (3 Litters)		Group *d* (3 Litters)		Group *e* (3 Litters)		Group *f* (2 Litters)	
	n	*x*	*n*	*x*	*n*	*x*	*n*	*x*	*n*	*x*	*n*	*x*	*n*	*x*	*n*	*x*	*n*	*x*
1			14	4 6			11	4 9							27	5 4		
2	12	5 0											35	5 3			21	4·8
3									12	7 9	12	8 7						
4									11	9 1	11	10 8			27	9 3		
5			14	8 1														
6	12	5 0					10	9 2										
7					5	13 7							35	9 9			21	12 4
8									11	13 6	11	16 5						
10	10	6 8							12	17 6	12	18 7						
11			14	15 1					11	17 0	11	21			27	19 3		
13	8	10 0																
14													34	19 3			21	23
15					5	29			11	20	11	24						
16	6	13 0																
17									12	29	12	25						
19			14	24											27	33		
20							10	29										
21					5	43					10	40	33	32				
22									10	32								
23	6	20									12	42					21	36
24									10	44								
25			14	37														

n, Number of pups
x, Mean weight in grams

There were severe atrophic changes in the thymus, spleen and lymph nodes

To examine the capacity for delayed hypersensitivity, healthy rats with no signs of runting syndrome in dietary groups *a–e* were immunised at 6 weeks of age with 0 05 ml of killed BCG bacilli 3 mg ml^{-1} in complete Freund's adjuvant Group *f* was immunised with 8 mg ml^{-1} *Mycobacterium tuberculosis* in heavy mineral oil Two weeks later animals were tested intradermally in a shaved flank with 250 tuberculin units of purified protein derivative (Connaught Laboratories) Diameter of induration was recorded 24 h later Results are shown in Fig 1 By median tests, the tuberculin reactions of the control groups did not differ significantly from each other and the skin test reactions of group *a* were significantly smaller than for the five control groups

I next investigated the effect of congenital pyridoxine deficiency on 25 Lewis rats in group *a* and 10 Lewis rats in group *c* Healthy non-runted rats of both sexes were immunised at 6 weeks of age with 0 05 ml killed *M tuberculosis* (8 mg ml^{-1} in heavy mineral oil) in one hind footpad and 0 05 ml complete Freund's adjuvant in the other hind footpad Tuberculin tests were done 2 weeks later The average diameter of induration for the 10 rats in group *c* was 7 3 mm and only one rat had no induration Fifteen of 25 rats in group *a* had no tuberculin reaction and the average diameter of the reaction was 2 2 mm The differences were statistically significant Work with Lewis rats was abandoned because of their low fertility

To study antibody responses, healthy male non-runted Sprague–Dawley rats in all six dietary groups were immunised intravenously with 10^8 sheep red blood cells at 6 weeks of age These animals had not been used in the tests for delayed hypersensitivity mentioned above Haemagglutination titres were determined at weekly intervals for 4 weeks on four rats in group *a* and 24 rats among the five control groups Four weeks after primary immunisation, all rats were given a second intravenous immunisation with

Table 2 Weights from 31 to 60 d of age of male non-runted rats whose dams were pyridoxine-deficient during gestation (group *a*) or were fed a normal diet (group *c*)

Age (d)	Group *a* Litter 1 Weight (g)	Litter 2 n	Litter 2 *x* ± s d	Litter 3 n	Litter 3 *x* ± s d	Litter 4 n	Litter 4 *x* ± s d	Group *c* (3 Litters) n	Group *c* (3 Litters) *x* ± s d
31								4	84 ± 9
32		6*	60 ± 7						
33						4	101 ± 10		
35				3	125 ± 12			6	91 ± 9
37	71†								
39		6	113 ± 5					12	129 ± 12
41						4	137 ± 13		
42				3	162 ± 12				
44	112								
45								12	166 ± 10
46		6	155 ± 8						
48						4	170 ± 18	6	130 ± 11
49				3	205 ± 15				
52								8	198 ± 9
53								4	197 ± 9
54		6	190 ± 12						
55						4	210 ± 18		
56				3	216 ± 18				
60		6	199 ± 10						

*A male runt weighed 37 g on day 32, 36 g on day 39, 33 g on day 46 and died on day 67
†A male runt weighed 36 g on day 44, the day of death

sheep red blood cells and haemagglutination titres were then determined at weekly intervals for 4 weeks Antibody responses in group *a* fell within the range of those of the control groups after both immunisations A similar result was obtained after primary and secondary intravenous immunisation with *Escherichia coli* of 4 male Sprague–Dawley rats of group *a* and 26 male rats of the five control groups Although the groups were small, the results were sufficient to show that congenital pyridoxine deficiency did not cause an obvious deficiency in primary or secondary antibody responses to these two antigens

At 8 weeks of age, when the congenitally pyridoxine-deficient rats exhibited a defect in delayed hypersensitivity, routine histological examination showed that the thymus, spleen and lymph nodes were in normal condition

These results demonstrate that congenital pyridoxine deficiency produces a defect in the capacity for expression of delayed hypersensitivity in 8-week-old rats The normal expression of delayed hypersensitivity in the five control groups excludes deficiency of other dietary factors or maternal protein-calorie malnutrition as causative factors How long the immunodeficiency persists remains to be determined

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B-cell suppression of delayed hypersensitivity reactions

DURING certain normal immunisation schedules, suppressor cells are generated and act in suppressing the expression of delayed hypersensitivity reactions[1,2] The suppression is immunologically specific and is dependent on living cells We have suggested that the suppressor cells were B cells because they are depleted when 300 mg kg^{-1} cyclophosphamide (CY) is given 3 d before sensitisation[3–5]

The most direct method of determining, with greater certainty, which lymphocyte subpopulation is responsible for the suppression of the expression of delayed hypersensitivity reactions is by using cell separation techniques Antibody coated immunoabsorbent (plastic bead) columns were therefore used to separate T and B lymphocytes

We used the same *in vivo* models as previously described for the assay of suppressor cells[1] Briefly, Hartley strain guinea pigs, treated with 300 mg kg^{-1} CY intraperitoneally (LD$_{10-20}$) 3 d before immunisation with 1 μg ovalbumin (OA) in Freund's incomplete adjuvant (FIA) given subcutaneously in the footpads, served as cell recipients 8 d later They were then potentially susceptible to the effect of suppressor cells which they were lacking In other experiments normal recipients were used Thrice washed pooled spleen cells (3×10^8 viable as determined by 0 2% trypan blue exclusion) were obtained from animals sensitised with 1 μg OA in FIA subcutaneously 8 d previously These cells were injected intravenously into the CY treated recipients which were skin tested, 1 h later, by an intradermal injection of 100 μg OA into the shaved flank Skin test reactivity was assessed as described previously[6] Spleen cells from the same pool were passed down plastic bead (Degalan V26) columns which had been coated with specifically purified rabbit anti-guinea pig γ globulin (R/GPγ) The R/GPγ was prepared in rabbits, which were sensitised to purified ($(NH_4)_2SO_4$ precipitation followed by three passages through DEAE cellulose columns) guinea pig γ globulin (GPγ) in Freund's complete adjuvant It was purified by binding to and elution from GPγ coated cyanogen bromide-activated sepharose beads (Pharmacia) The method of Campbell and Grey was used to coat the plastic beads[7] A flow rate of 2–2 5 ml min^{-1} was used and approximately 2×10^9 spleen cells (viability 50–60%) were applied to each of the 30×1 5 cm columns There was a 12–20% recovery of cells from each column A direct immunofluorescent (IF) technique using either FITC labelled goat or R/GPγ (Nordic) was used to identify B cells The effluent cells were pooled and found to be depleted of 90–95% of the 27–36% of IF positive cells which were present in the original cell suspensions applied on to the columns Effluent cells did not stain with FITC-labelled goat anti-rabbit γ globulin which indicated that the cells were not coated with R/GPγ when they were passed through the columns Plastic bead columns coated with normal rabbit serum (NRS) were used in control experiments Since one-third of the 3×10^8 viable donor cells were IF-positive in the original suspensions, we transferred 2×10^8 viable effluent (B-cell depleted) cells into the CY treated recipient animals and assayed their ability to suppress the delayed hypersensitivity reactions

Table 1 Skin reactivity of animals, treated with CY 3 d before sensitisation with 1 μg OA in FIA, serving as cell recipients 8 d after immunisation

Donor cells†	No of recipients	Increase in skin thickness* 24‡	48	72
Control§	11	4 4±0 74	5 6±1 04	4 0±1 10
Unseparated‖	5	1 8±0 27††	2 2±0 27††	1 4±0 89††
Separated¶ (R/GPγ column)	6	4 6±0 58	5 4±0 86	4 3±1 29
Unseparated** (NRS column)	5	2 1±0 65	3 1±0 89	2 0± 0 79

*10^{-1} mm ± s d
†Pooled spleen cells from animals immunised with 1 μg OA in FIA 8 d earlier
‡Time after intradermal skin test with 100 μg OA
§Received no cells
‖3×10^8 viable, containing 27–36% IF-positive cells
¶2×10^8 viable, containing < 3% IF-positive cells
**3×10^8 viable, containing 26% IF-positive cells
††$P < 0 001$ when compared to control animals and separated cell recipients using Student's *t* test

Table 1 shows that cells that were not passed through the columns suppressed reactions of the recipient CY-treated animals to a similar extent as previously described[1] When cells depleted of 90–95% of B cells (after passage through R/GPγ coated columns) were transferred into identically treated recipients, however, there was no suppression of the delayed-in-onset skin reactions (Table 1) The cells passed through NRS coated columns contained 26% B cells (compared to 28% in the original cell suspension) and suppressed delayed reactions of the CY-treated recipients to a similar degree as the unseparated cells (Table 1)

We had previously demonstrated that the passive transfer of spleen or PEC from animals sensitised to 1 μg OA in FIA 8 d earlier caused more intense and more indurated reactions in normal recipients when donors had been pretreated with CY 3 d before sensitisation, as compared to those reactions transferred from sensitised animals which had not received CY (ref 1) We therefore passively transferred spleen cells, which had been depleted of B cells (as above), into normal guinea pigs to determine whether they would also transfer the more intense and more indurated reactions Table 2 shows that 2×10^8 viable column-separated, B cell-depleted, spleen cells from animals sensitised with OA in FIA 8 d earlier caused more indurated reactions than the 3×10^8 viable unseparated spleen cells from the same cell pool We favour the interpretation that B cells are suppressing the expression of delayed hypersensitivity reactions because after removing B cells, the same number of transferred non-B cells were causing more indurated reactions in normal recipients but no longer suppressed the skin reactions in the CY-treated sensitised recipients

Table 2 Skin reactivity of normal recipients receiving pooled spleen cells from animals immunised with 1 μg OA in FIA 8 d earlier

Donor cells	No of recipients	Increase in skin thickness* 24†	48	72
Unseparated‡	6	2 4±0 58	0 42±0 58	0
Separated§	5	3 9±0 65‖	2 3±0 57‖	1 0±0 61‖

*10^{-1} mm ± s d

†Time after intradermal skin test with 100 μg OA

‡3×10^8 viable, containing 27–36% IF-positive cells

§2×10^8 viable, containing < 3% IF-positive cells

‖$P<0\ 001$ when compared to control animals and separated cell recipients using Student's *t* test

These experiments demonstrate that, after immunisation with OA in FIA, B cells or their product(s) suppress the expression of the delayed hypersensitivity reactions These reactions are undoubtedly T-cell functions because, in the absence of B cells, they are more indurated and more intense Other recent studies have also demonstrated B-cell suppression of T-cell functions[8–10] It should be mentioned that numerous published studies have demonstrated that T cells may amplify, as well as suppress, other T cell functions[11–14] It seems therefore that B cells, as well as T cells, are important in the homeostasis of T-cell functions

The mechanism of the suppression of T-cell functions by B suppressor cells remains unknown We, as well as others cited above, have been unable to implicate the antibody product of B cells in the mechanism of the suppression It could be that the suppressor cells in delayed hypersensitivity form a separate subpopulation of easily identifiable Ig-coated cells that is distinct from those which become antibody producers Effective methods for recovering pure B cell populations should help elucidate the mechanism(s) involved in this suppression

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New method for extending the diffraction pattern from protein crystals and preventing their radiation damage

A CONSIDERABLE problem hampering the analysis of protein crystal structures has been the damage caused to the crystals by the X-ray beam Another limiting factor has been the resolution in the diffraction pattern To increase the useful life of a protein crystal in the X-ray beam[1], single crystal monochromators and low temperatures have been used We now report that styrene and other vinyl monomers can decrease the radiation damage of the crystal in the X-ray beam by a factor of as much as 10, and extend the diffraction pattern so that higher angle reflections can be measured

We used crystals of an intact IgG immunoglobulin molecule (DOB) whose crystal structure has been determined to a resolution of 6 Å (ref 2) These crystals are extremely sensitive to radiation, and intensities of even very low order reflections with d spacings greater than 8 Å decrease to 80% of the starting value in about 15 h of exposure The diffraction pattern does not show any meas-

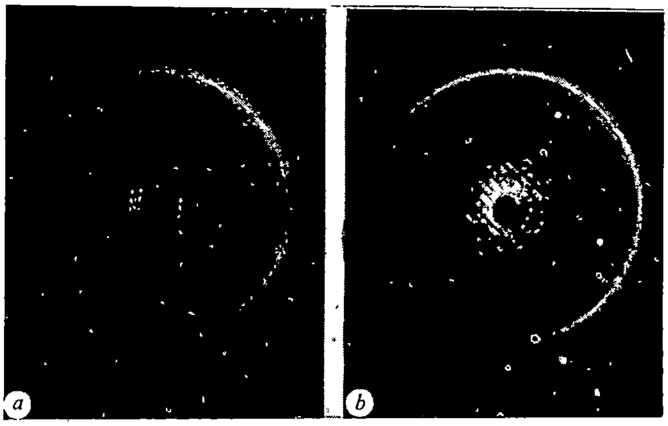

Fig 1 Diffraction pattern from (*a*) an untreated and (*b*) styrene-treated immunoglobulin crystal The precession angle is 15°, exposure time 45 h Both crystals were of comparable size The X-ray beam was parallel to the *b* axis

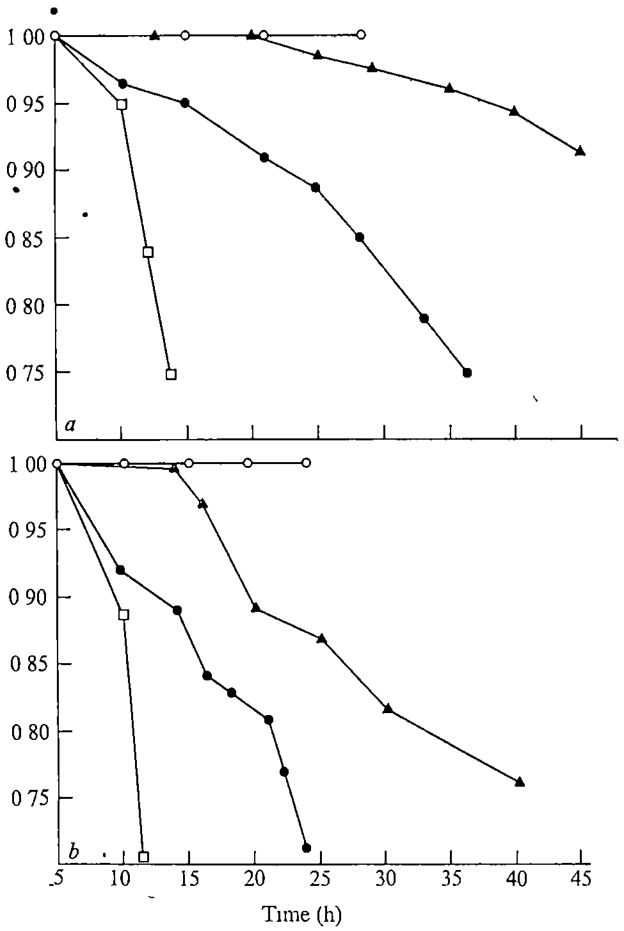

Fig 2 Variation of I_t/I_0 with exposure time for two reflections (0 8 0) (*a*) and (−12 0 6) (*b*) with the following styrene concentrations ●, control, ○, 2 0 mM, ▲, 1 2 mM, □, 30 mM These are styrene concentrations in the protein solution at the start of crystallisation The actual concentration of styrene inside the crystal lattice is unknown I_0 corresponds to the background corrected intensity at time=5 h A single crystal monochromator was used on the diffractometer and all measurements were carried out at room temperature

urable reflections beyond a resolution of about 5 5 Å (ref 3) (Fig 1*a*)

These crystals are usually grown at 4° C in 0 1M sodium borate buffer at *p*H 8 8 We have grown the same crystals in the presence of various amounts of concentrated styrene monomers added to the protein drop before it is set for crystallisation When irradiated, the treatment crystals continued to diffract even after 150 h in the X-ray beam showing sharp reflections that extend to a resolution of 4 Å (Fig 1*b*) Due to increased error involved in adding less than 0 5 μl of styrene directly to the protein drop, we also crystallised the immunoglobulin by adding different amounts of the buffer saturated with styrene

Figure 2 shows the variation in intensity with time of exposure of two arbitrarily chosen reflections from an untreated crystal and crystals treated with styrene Data were collected using a Nonius CAD4 automatic diffractometer Table 1 shows the intensities of a typical row of reflections with decreasing interplanar spacings from two control crystals of different dimensions and one treated crystal The intensities from the treated crystal were greater than those from the control cyrstal even when the latter had a volume 50% greater than the former This effect was particularly noticeable with the higher order reflections

We have diffused styrene monomers into pregrown immunoglobulin crystals by placing a drop of the concentrated styrene adjacent to the protein drop in a sealed well

Table 1 Intensity values for a typical row of reflections with decreasing of spacing

h k l	d(Å)	2 mM styrene treated (I_s)	No styrene control 1 (I_1)	No styrene control 2 (I_2)	I_s/I_1	I_s/I_2
9 1 0	20 3	9,287	2,931	6,299	3 1	1 5
11 1 0	16 8	11,054	3,335	7,183	3 3	1 5
13 1 0	14 3	3,819	1,355	2,226	2 8	1 7
15 1 0	12 4	6,497	1,935	3,406	3 3	1 9
17 1 0	10 9	190	0	0	∝	∝
19 1 0	9 8	291	18	141	16 0	2 0
23 1 0	8 1	280	82	142	3 4	2 0
25 1 0	7 5	375	0	223	∝	1 7
29 1 0	6 5	1,390	410	703	3 4	2 0
31 1 0	6 0	613	177	288	3 5	2 1
33 1 0	5 7	199	45	70	4 4	2 8
35 1 0	5 4	233	53	78	4 4	3 0
37 1 0	5 1	504	0	156	∝	3 2

The treated crystal had dimensions 0 8 × 0 5 × 0 15 mm The control crystal 1 had dimensions 0 7 × 0 5 × 0 15 mm, comparable with the size of the treated crystal The control crystal 2 was larger than the treated crystal and has dimensions 1 0 × 0 6 × 0 15 mm The increase in the ratio for the high order reflections suggests a lowering of the temperature factor for the treated crystal The zero intensities represent those reflections for which the peak to background ratio was less than one A single crystal monochromator was used on the diffractometer and all measurements were carried out at room temperature

Results similar to those described above were obtained, but the concentration of the styrene in the protein drop was not so well determined as in the cocrystallisation experiments We have also used monomers such as chlorostyrene, and methyl methacrylate with similar results We have not used any styrene-treated crystals for preparing isomorphous heavy atom derivatives

We propose to calculate a difference electron density map between the treated and the untreated crystal to locate regions of styrene binding in order to understand the actual mechanism of the protection provided by styrene We speculate that styrene monomers are trapped in the intermolecular volume of the crystal lattice and are polymerised by the X radiation The aromatic nucleus in styrene is a well known energy trap[4] and presumably scavenges the free radicals formed in the crystal, thereby reducing its radiation damage We also suggest that at low styrene concentrations, a polystyrene cage surrounds regions of the protein molecule limiting its movement, thus extending the diffraction pattern But with very large concentrations of styrene, the rapid polymerisation combined with the increased concentration of polymer presumably gives rise to large disordered regions in the protein molecule thus worsening the observed diffraction pattern

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Do immunologically privileged sites require a functioning spleen?

WHEN solid tissue allografts are transplanted orthotopically to alien hosts they are rejected with a characteristic tempo and vigour that depends primarily on the immunogenetic disparity between donor and host There are, however, several anatomical sites in which prolonged graft survivals have been documented These so-called immunologically privileged sites include the brain[1,2], anterior chamber (AC) of the eye[3–5], cheek pouch of the Syrian hamster[6] and artificially created skin pedicle flaps in guinea pigs and rats[7,8] There is considerable experimental evidence that these sites share at least one common feature—absence of a demonstrable lymphatic drainage[7–9] As a consequence, it has been assumed that the putative immunological privilege of these diverse sites results from the anatomical aberration which interrupts the afferent limb of the immunological reflex arc (which is composed of an afferent limb, a central mechanism and an efferent limb) We have now found that allogeneic cells placed within the AC of the eye are not isolated from the immunological apparatus of the host, but instead induce a primary immune response within the spleen which alters the systemic immunological response of the intact animal

To determine the status of a lymphatic drainage route from the AC, we used a local graft-*versus*-host lymph node assay Lymph node cells from Fischer rats specifically sensitised to tissue antigens of DA strain rats were inoculated into either the AC or the subconjunctival space (SCS) of (F×DA)F_1 recipients Eight days later (Table 1) hypertrophied cervical lymph nodes developed in response to SCS injections, whereas local lymphadenopathy did not follow AC injections of donor cells This provided strong circumstantial evidence that the AC lacks lymphatic drainage

Table 1 Comparison of cervical lymph node hypertrophy after AC or SCS inoculation of allogeneic lymphoid cells

Route of inoculation	No of animals	Experimental*(mg)†	Control	*P*
Subconjunctival space	10	237 2	84 2	<0 005
Anterior chamber	22	88 0	89 4	>0 20

*Lymph nodes to be weighed were removed from (F × DA)F_1 hybrid hosts 8 d after the inoculation of 10 × 10^6 F anti-DA lymph node cells into the anterior chamber or subconjunctival space

†The weights of the superficial cervical lymph nodes, that is the submandibular and facial nodes are reported

Slit lamp microscopy showed that after being placed in the AC the inoculated cells disappeared within a few days by an unknown route These cells reached the systemic circulation and made a significant impact on the host's immunological apparatus, as our next series of experiments showed In the first, genetically tolerant (F×DA)F_1 lymphoid cells were inoculated into the AC of normal Fischer rats whose sera were regularly tested for haemagglutinating activity directed at DA alloantigens Surprisingly, anti-DA antibodies were indentified as soon as 4 d after inoculation and persisted for at least 10 d thereafter (Fig 1) In the second series of experiments, panels of Fischer animals were inoculated similarly with (F×DA)F_1 lymphocytes into the AC and challenged orthotopically with (F×DA)F_1 skin allografts 4, 7, 10 and 14 d later During the second week after inoculation the animals exhibited significantly prolonged survival of (F×DA)F_1 hybrid skin grafts (Fig 2) The median survival time (MST) of F_1 skin grafted 10 d after inoculation was 12 0 d, with some grafts surviving 14 d Since first set (F×DA)F_1 skin grafts were rejected by normal hosts with a MST of 9 1 d, the prolonged MST in the experimental group during the second week is highly significant It is interesting that F_1 skin grafts applied during the first week after inoculation had a MST similar to that of first set grafts The similarity of MSTs for first and second set skin allograft rejection in rats with an AgB histoincompatibility makes it very difficult to distinguish between the two Consequently, it is possible that during the first week after inoculation of F_1 lymphoid cells into the AC, some recipients were sensitised specifically without its being revealed by the gross test of graft rejection

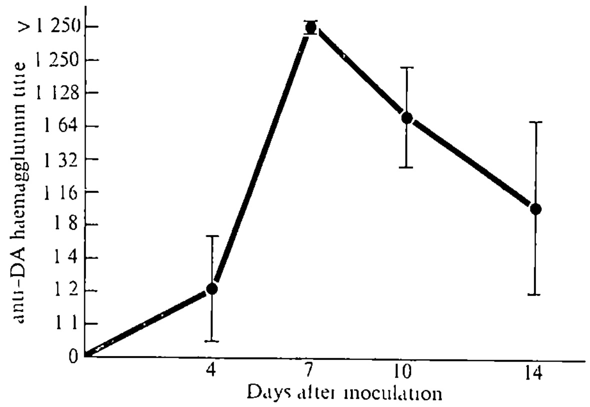

Fig 1 Anti-DA haemagglutinin antibody titres after the inoculation of 10 × 10^6 (F × DA)F_1 lymphoid cells into the AC of Fischer hosts ($n=6$) Bars represent one standard deviation

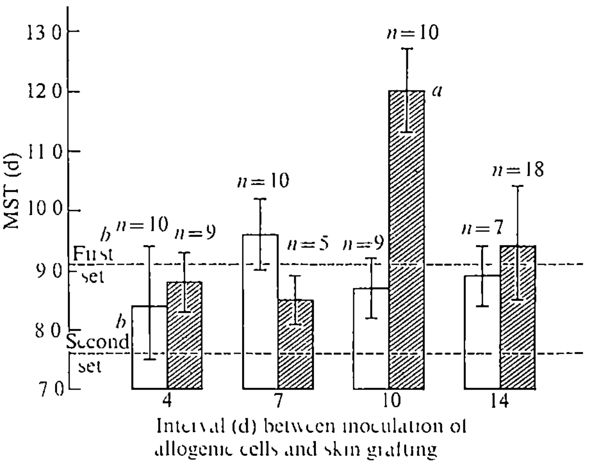

Fig 2 The comparative MSTs of (F × DA)F_1 hybrid skin grafted within the first 2 weeks after inoculation of 10 × 10^6 (F × DA)F_1 LNC into the AC (hatched columns) or subconjunctival space (open columns) of Fischer hosts The 95% confidence limits are represented *a*, The MST of F_1 skin allografts 10 d after inoculation of allogeneic lymphoid cells into the AC is statistically significant from a first set reaction, using Student's *t* test, with $P < 0.001$ *b*, MSTs of first and second set (F × DA)F_1 grafts in normal Fischer hosts are presented for comparison

By contrast, grafts of F_1 origin placed on Fischer hosts that had been inoculated subconjunctivally with (F×DA)F_1 lymphoid cells were rejected abruptly irrespective of the time between inoculation and graft application (Fig 2)

Only intravenous antigen presentation gave results similar to those obtained with injection into the AC (Fig 3)

Since the AC lacks lymphatic drainage, and since our data suggest that alloantigens on lymphocytes placed in the AC made an impact on the host's immunological apparatus, we suggest that donor lymphoid cells escaped from the AC directly into the blood vasculature of the host By virtue of its size and blood flow, the spleen receives the majority of antigen presented to an animal intravenously[10–12] Moreover, the spleen has been identified as a major source of enhancing antibodies[13] Consequently, it seemed reasonable to determine whether the facilitation of skin graft survival displayed by Fischer hosts during the second week after inoculation of (F×DA)F$_1$ cells, by the AC or intravenous routes, depended on an intact, functioning spleen Accordingly, Fischer hosts were splenectomised and after 28 d were inoculated, into the AC or intravenously, with (F×DA)F$_1$ lymphocytes Challenge with skin grafts of (F×DA)F$_1$ origin (Fig 3), revealed no evidence of enhancement or facilitation, in fact, the MST of these grafts (8 3 and 7 8 d respectively) approached the accelerated rejection usually found in specifically sensitised hosts (7 8 d) These data suggest that the spleen is an essential component of the immunological mechanism that temporarily subverts cell-mediated responses

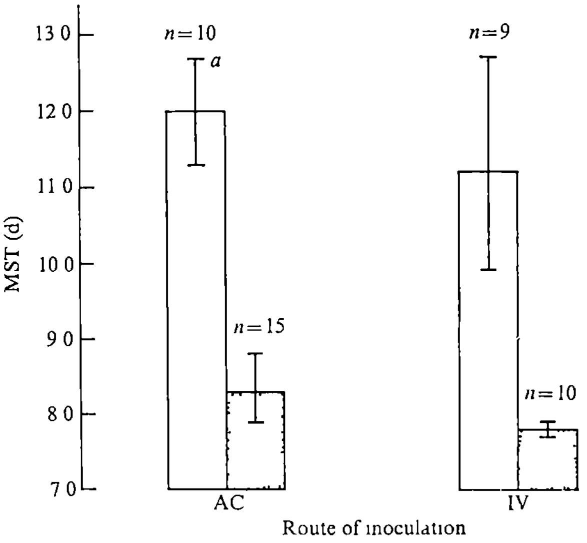

Fig 3 A comparison of the MSTs of (F×DA)F$_1$ hybrid skin grafts in normal (open columns) and splenectomised (dotted columns) Fischer hosts after the inoculation of 10×10^6 (F×DA)F$_1$ lymphoid cells into the AC, 10 d before grafting, or intravenously, 7 d before grafting The shortened survival time of allografts on splenectomised hosts, after both AC or intravenous injection of antigen, is highly significant compared with normal hosts ($P < 0 0005$) *a*, The 95% confidence limits are represented

Skin grafts placed within the AC exhibit survival patterns significantly different from orthotopically placed grafts, which cannot be attributed solely to a delay in host sensitisation[4,20] We propose that although all immunologically privileged sites appear to lack demonstrable lymphatic drainage routes[7–9 14], the critical feature for at least some of them is the ability to allow antigen direct access to the blood vasculature without first encountering a peripheral lymph node As a consequence, the host's initial antigen exposure occurs predominantly within the spleen and our results indicate that the microenvironment of this initial encounter significantly influences the nature of the subsequent immunological response Our data do not reveal which components of the spleen give it the special properties we have observed But, together with other results[15–19], they suggest that one immunological function of the spleen is to divert what would otherwise be essentially a cell-mediated and tissue destructive response into an antibody-mediated response which is often protective of the same target tissue It seems reasonable to speculate that the spleen achieves this conversion by deflecting potential effector T lymphocytes (killers) into cooperative interactions (helpers) with appropriate B cells An effective cell-mediated response is thus thwarted by (1) a temporary reduction in the number of circulating killer cells and (2) an enforced competition between the remaining killer cells and enhancing antibodies directed at the same target tissue

It is conceivable that this immunoregulatory role of the spleen in the induction of the immune response represents the evolutionary advantage of vertebrates in coping swiftly and successfully with certain kinds of hazardous microbial infections[21–24] By the same token, however, it may place the host at considerable disadvantage with regard to the capacity to control aberrant and neoplastic growth for which cell-mediated immune processes are crucial

We thank R E Billingham for helpful discussions and critical review, R Zeppa for support, and R G Holt for technical assistance This work was supported by a National Institutes of Health research grant and research training fellowship

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reviews

Some corner of a foreign field

by R. V. Jones

H G J Moseley The Life and Letters of an English Physicist, 1887–1915 By J L Heilbron Pp viii+312 (University of California Berkeley, Los Angeles and London, June 1974) £7 50

"I BEG to apply for the Poynting Professorship of Physics I have been nominated for and hope to obtain a temporary commission in the regular army preferably in the Royal Engineers My services are not therefore likely to be available while the war lasts " So wrote H G J Moseley, then 26 years old, to the University of Birmingham on October 8, 1914 On August 10, 1915, he was killed in a desperate action against the Turks in Gallipoli

With a father and grandfather both Fellows of the Royal Society, with an Eton and Oxford education and a private income, Moseley was, as his American biographer shows, a very English physicist In his short life he had already accomplished one of the most brilliant pieces of experimental physics of this century, and he was a hero to the schoolboys of my generation who learnt, as E J Holmyard put it in his texbook of Chemistry (quoting Soddy), that Moseley had "called the roll" of the chemical elements and put them all in their place

Moseley's example was a comfort, too, to the undergraduates of my day, for he had taken only Second Class Honours in Physics Finals at Oxford At an Oxford Physical Society dinner in 1931 I was sitting next to our Professor, J S E Townsend, and asked him whether this was so, Townsend told me how indignant he had been that Moseley had been given a Second, and said that if he had known that Moseley was ill (from hay fever) he would have remonstrated with the examiners before the results were published

That was in 1910, after finals Moseley went to Manchester, where he had been attracted by the brilliance of Rutherford The latter put him on to determining the number of electrons emitted in the decay of radium, a task which he completed in April 1912 In that same month, Friedrich and Knipping, prompted by von Laue, discovered the diffraction of X rays, and the work was taken up in Leeds by the Braggs Barkla had already discovered that elements under election bombardment emitted two characteristic rays, one harder than the other, which he designated K and L, and Whiddington had found in 1911 that the velocity which cathode rays must have to excite K radiation in an element of atomic weight A is about $A\times10^8$ cm s^{-1} With the debate going on between the Braggs, von Laue and Barkla concerning the nature of X rays, Moseley became interested, and together with C G Darwin he started to experiment with them (But could he have used a mercury vapour pump in 1912? (page 73)—Gaede did not describe such a pump till 1915)

Moseley's skill with apparatus quickly resulted in his recognition of X-ray wavelengths as characteristics of atomic structure and, as he wrote to his mother in May 1913, "there is here a whole new branch of spectroscopy, which is sure to tell one much about the nature of an atom " He was soon recording the X-ray spectra of various elements, and by the following November he had found that the frequency of the K_α line is given by

$$\nu_{K\alpha}/\nu_0=(3/4)(Z-1)^2$$

where ν_0 is the Rydberg frequency already known from optical spectroscopy, and Z the atomic number, and he equated the atomic number with nuclear charge which he recognised as chemically more significant than atomic weight At that point he returned to the Electrical Laboratory in Oxford, where he completed the work on some 30 elements and was able to put all these elements in order, including some hotly disputed rare earths, simply by determining the atomic numbers from the K_α frequencies Moreover, he could show exactly where any gaps remained to be filled in the Periodic Table, and he could make sense of those few elements which, judged by their atomic weights, had seemed to be out of place

By 1914 Moseley was interested in the prospectively vacant chairs in Oxford and Birmingham, but war broke out just as he was about to land in Australia for the British Association meeting He made a rapid return to England with Henry Tizard after the meeting, both to volunteer for active service They left ship at San Francisco to cross America by rail, and ran to catch the Lusitania in New York, arriving in England on September 28 After a period of military training with the Royal Engineers (he had been in the OTC at Oxford) Moseley volunteered for the Royal Flying Corps, but his transfer was vetoed, and he was sent to Gallipoli

On the way out he made a will bequeathing all his interests to the Royal Society "to be applied to the furtherance of experimental research in Pathology, Physics, Physiology, Chemistry, or other branches of science but not in pure mathematics, astronomy, or any branch of science which aims merely at describing cataloguing or systematizing" This bequest formed the basis of the Moseley Studentship, whose second holder was P M S Blackett

Moseley had been one of the 15 or so King's Scholars elected to Eton in 1901 six of them "including all who were natural leaders in work or play" were killed in the war Moseley's death, recognised even in Germany for the loss it was to science, was more than any other event instrumental in our wiser use of scientific talent in the Second World War

The story of Moseley's life is admirably told by Professor Heilbron, who has been to great pains to gather Moseley's letters and to investigate many private papers of his contemporaries The letters are both piquant and poignant they give impish sidelights on figures such as Hugh Dalton, Harold Hartley, Julian Huxley, Lindemann, Rutherford, Tizard and Townsend, and show both the attractions and defects of Edwardian Oxford with its overpowering classical complacency As Professor Heilbron speculates in his epilogue, the development of physics at Oxford would probably have been different if Moseley had survived, for he would probably have been preferred over Lindemann, and he would have been more active in the laboratory and a Nobel prize winner

Though physics is no medium for nationalism, the sub-title of this volume is significant for more than the fact that in connection with Moseley it must recall the lines of *The Soldier* by his contemporary Rupert Brooke, who was also to die in the Gallipoli campaign For Moseley himself wrote to Rutherford "the French point of view [of physics] is essentially different from the English Where we try to find models or analogies, they are content with laws" But irrespective of whatever truth there may have been in this comment, physicists of all nationalities who are interested in the development of their subject owe Professor Heilbron a debt for his excellent biography And if this review seems to have been coloured by Moseley's own opinion that "reviews in *Nature* are usually such pure honey", in this case both the subject and his biographer deserve it

Evolution of populations

Genetical Structure of Populations By Kenneth Mather Pp viii + 197 (Chapman and Hall London, 1973) £4 00

Professor Mather's latest book is a survey of topics in population genetics, intended for advanced undergraduate and postgraduate students seeking a general orientation The subject is the way in which proportions of genetic types change from generation to generation in response to various factors, and the effect of these changes on the status of the population in relation to its environment The approach is quantitative but not mathematical, being directed to readers with biological education and interests The mathematical level varies considerably from chapter to chapter with more extensive algebraic treatment of traditional topics such as the Hardy-Weinberg law and its generalisations

After a short introduction the style of the work is established in the second chapter which reviews the causes of viariability in biology Professor Mather assumes that readers understand the basic facts of inheritance, and devotes himself to a detailed exposition of the way in which these affect population changes Although the results of algebraic analysis are stated, most proofs are omitted Later in the book, chapter 6 on "Life Cycles and Genetic Systems" is a mine of interesting information but contains no mathematical analysis at all Chapter 7 on "Genic Balance and Genetic Architecture" involves quantitative statements about the population effects of the manner in which the genetic system acts on numerical characteristics, but again contains no proofs

Chapters 3 and 4 ("Equilibria" and "Theory of Variability") reveal the strength and weakness of the attempt to discuss a quantitative subject while eschewing advanced mathematics In mathematical books on population genetics (among which Moran's *Statistical Processes of Evolutionary Theory*, published in 1962 remains unsurpassed despite many subsequent competitors) the problem of selection, random tendency to fixation, mutation, non-random mating and so on, are treated as separate topics In nature they are all acting simultaneously, one reinforcing or counteracting the effect of another Professor Mather makes this clear by detailed examples in a full and well balanced discussion This chapter leaves the reader with a profound respect for the author's deep knowledge of what is really going on in a natural population and a realisation of how much can be lost through oversimplification

Two points of substance are worthy of note It is clear towards the end that Mather believes that competition is always frequency dependent, and it must follow that much recent work in mathematical genetics is of little value since it assumes fixed selection coefficients He devotes several pages to a discussion of the concept of 'genetic load' which has been so hotly debated in recent years Mathematicians with an interest in genetics, who have been worried by their inability to see the point of all this, will be pleased that such a distinguished geneticist concludes "it is far from clear that the concept, as it has been defined and used, has much significance at all for the understanding of populations and their structure"

In discussing the theory of variability, Professor Mather applies a statistical analysis of variance to the variation in a numerical trait in a population He supposes, however, that it contains equal proportions of the alleles at one locus or at each of two loci, and obtains components of free, potential and utilised variability which can be transformed into one another as a result of various effects and which are "acted on" by the factors of selection These ideas clearly give insight into the heart of Mendelism, the fact that variability provides the basis for evolution They would explain it completely if, when the gene proportions are not equal, the transformation of components behaves qualitatively in the same way Now in the case of constant selection depending on two or more loci this is not true There may be several stable equilibria, each with its domain of attraction, and it is unlikely that the same analysis of the responses of components of variability to selective factors will hold in the neighbourhood of them all The analysis is therefore too restricted in its validity to be convincing The weaknes of this chapter and the previous one is the readiness with which it is assumed that the qualitative results of algebra applied to simple systems can be extended to complex systems for which results have not been obtained

This has led to wrong conclusions in the past, and the student should be warned against the practice rather than led into it It may well be that when it becomes possible to analyse fully realistic models, the results will prove to be on the lines described by Professor Mather on the basis of his wide knowledge, since factors neglected in (say) the multilocus constant selection model may then appear and eliminate superfluous stable equilibria It is also probable that a satisfactory mathematical analysis of these problems cannot be achieved through classical algebra and will require the application to genetics of topological considerations such as those introduced by Thom in biology

This is an admirable textbook for students, containing a masterly exposition of what is really important in our current knowledge of population genetics In order to go beyond it a student will need to study the mathematical side of the subject at a much higher level, and should be encouraged to regard at least some of the assertions as tentative until they have been proved

Philip Holgate

Reproduction of marine invertebrates

by R. P. Dales

Reproduction of Marine Invertebrates Vol 1 Acoelomate and Pseudocoelomate Metazons Edited by Arthur C Giese and John S Pearse Pp xi+546 (Academic New York and London, 1974) $38, £13 45

This useful and scholary work is the first of seven volumes The first six will give a systematic treatment with a chapter devoted to each class or phylum, the final volume will be devoted to general aspects of reproductive physiology This first volume is especially useful for its coverage of so many minor but interesting phyla such as Kinorhynchs and Gnathostomulids But all of the chapters are useful I found the amount of descriptive detail to have been well judged and the accounts of reproduction and embryonic development to be concise and informative The appearance of the series is timely for there has been much recent work on reproduction in invertebrate phyla

References are given in full at the end of each chapter and the volume as a whole is provided with separate indices to authors, subjects and taxonomic names The volume, containing many line figures and half-tones, is well produced No zoological library can afford to be without this series

Dangers of lead

by B. E. Clayton

Sub-Clinical Lead Poisoning By H A Waldron and D Stoffen Pp x+224 (Academic London and New York, May 1974) £5 50, \$14 25

THE authors define sub-clinical lead poisoning as the production of morbidity or mortality without the appearance of the classical signs or symptoms of lead poisoning This is a highly emotive subject and the authors are to be congratulated for dealing with it in a balanced and scientific manner They have provided an excellent review of the literature including that from eastern Europe, and over 1,000 references are given together with an author's index

The book is divided into six main sections lead in the environment, lead metabolism, pathological effects of lead, sub-clinical effects, diagnosis and lastly prophylaxis and treatment With such a controversial subject it is not surprising that I do not agree with all the statements made but the authors' main conclusion would be accepted by most workers They consider that more studies are required before it is possible to assess whether or not excessive exposure below that causing clinical lead poisoning is producing harmless physiological or psychological effects, especially in children

The book is well produced The list of references would have been even more valuable if titles of papers had been given An appendix listing quantities of lead in food and drink is included It is written clearly and workers from various disciplines would find it valuable I recommend it to all those concerned with this difficult environmental problem

Natural death

Biological Control By C B Huffaker Pp xix+511 (Plenum New York and London, 1974) \$11 75

IN recent years, increasing concern at the possible pollution of the environment by chemical pesticides has directed attention back to studies of non-chemical methods of pest control Before DDT and other efficient synthetic insecticides were brought into general use in the 1940s, control of pests by biological means, particularly by parasitic or predatory insects, had resulted in many successes Some of these, like the classical case of the Australian ladybird *Rodalia cardinalis*, which, in 1877, controlled the cottony cushion scale *Icerya purchasi*, have never been bettered, but there have been many other successes, some partial control, and some complete failures Nevertheless, as F J Simmonds has shown, the investment in research was so paltry, and the results in increased crops were so great, that the whole exercise, on a world scale, was extremely cost-effective The trouble was often that the individual who gained could not easily be implicated in the costs of the control Commercially produced chemical pesticides, on the other hand, could bring their financial rewards to industrial concerns

This paperback reprint of the proceedings of a conference, aims at making up to date information about biological and other methods of pest control available to a wider audience, and particularly to university students of entomology, agriculture and allied subjects If read critically, it should form a useful text

Nobody doubts that, particularly when improperly used, some agricultural chemicals can endanger wildlife the environment and even man himself We would all like to reduce the use of dangerous chemicals, and to use non-polluting methods of pest control But, unfortunately, some of the authors whose articles are reprinted here tend to overstate their case Thus the editor, C B Huffaker, says in his preface "employment of synthesised chemicals for insect pest control has posed an enormous and as yet unfathomed contribution to the degradation of our environment" This, and other similar statements, conceal the fact that over ninety per cent of pesticide usage throughout the world is effective, in that it achieves its object and no measurable damage can be demonstrated Yet we are clearly right to worry about potential dangers, and to try to find more effective controls

This book differs from previous texts on biological control in that although it describes once more all the well known successes, it includes a section on the ecological background to the subject and also devotes considerable space to 'integrated control' or what is now often called 'pest management' G C Varley and G R Gradwell deal with the fundamental basis in population ecology in their paper "The Use of Models and Life Tables in Assessing the Role of Natural Enemies" An understanding of these matters is obviously essential if biological control is to cease to be a hit and miss affair Unfortunately, these authors are still unable to demonstrate a single case where theoretical or mathematical studies have given any practical help to those engaged in practical control measures The inspired hunch has, on the other hand, often paid off It is to be hoped that this will not discourage others from these fundamental studies, which are necessary long-term—and which, it must be accepted, may never produce the desired results

The main lesson the book teaches is that put forward many years ago by the late W E Ripper that is that an "insecticide should be used like a rapier, not like a bludgeon" This is the essence of integrated control—natural enemies of pests should be encouraged but when they fail the correct chemical may be carefully used Also we are now learning that total pest eradiction is neither essential nor desirable Pests can often be 'managed' to prevent patent crop damage, and, at the same time, so as to ensure the survival of their natural enemies All these techniques will undoubtedly increase in importance, particularly if premature bans on allegedly dangerous chemicals continue to be introduced in some countries But the farmer, and even more the medical entomologist, will rely mainly on chemicals for many years to come It is right, however, that the disadvantages as well as the gains from chemical control should be recorded, though few writers can yet take a really objective view of the situation KENNETH MELLANBY

1975 Catalogue of

SCIENTIFIC & TECHNICAL JOURNALS FROM THE U.S.S.R.

on

GEOLOGY, GEOPHYSICS, BIOCHEMISTRY, BIOPHYSICS, NATURAL SCIENCES

and related subjects
(in Russian but many with summaries in English)
may be obtained from

Collet's

Denington Estate
Wellingborough, UK

obituary

B. L. Astaurov

ACADEMICIAN BORIS L'VOVICH ASTAUROV, the eminent Soviet geneticist, died on June 21, 1974

Astaurov was born in 1904, and was educated at the University of Moscow, where he studied under S S Chetverikov, the founder of the Soviet school of experimental genetics Shortly after graduation, he began working on the heredity and genetics of silkworms, completing his first major work in 1937 A convinced anti-Lysenkoist, whose career suffered considerably during the years of Lysenkoism, Astaurov became a focus for the anti-Lysenkoist reaction His 60th birthday party became a celebration of the fall of Lysenkoism and Astaurov played a significant part in the rebirth of Soviet genetics He was elected President of the 'Vavilov' All-Union Society of Geneticists and Breeders in 1966

M. Reinhard

PROFESSOR MAX REINHARD, a leading authority on Feldspars, died on August 17, 1974 He was 92

After acting as an assistant to Professor Duparc at the University of Geneva, he was appointed to the Chair of Mineralogy and Petrology at the University of Basel in 1923 He became Rektor of the University in 1943 and retired in 1952 with the title Professor Emeritus

Announcements

Miscellaneous

United States Public Health Service International Postdoctoral Research Fellowships Up to six awards may be made to Australians for research training in 'health-related' fields in the USA Applications to the Executive Secretary, Australian Academy of Sciences, PO Box 216, Civic Square, ACT 2608, Australia

The Worshipful Company of Scientific Instrument Makers/MIT Travelling Fellowship The Fellowship is open to a suitably qualified graduate with appropriate experience who must be a British national Full details and application forms are obtainable from the Clerk of the Worshipful Company of Scientific Instrument Makers, Alliance House, 12 Caxton Street, London W1H 0QY

International meetings

October 14–18, **Air Quality Management Systems,** Milan (Richard Tudway, International Institute for the Management of Technology, 59–63 Corso Magenta, Milan, Italy)

October 24, **Natural Products in Aquatic Environments,** London (Dr John F Gibson, The Chemical Society, Burlington House, London W1V 0BN)

Corrigendum

In the article "Exchange of neurotransmitter amino acid at nerve endings can stimulate high affinity uptake" by G Levi and M Raiteri (*Nature,* **250,** 735, 1974) the sentence at lines 21–22, p 737 should read Net uptake, which may or may not have a high affinity component

Errata

In the article "Interaction of coherent electromagnetic waves with relativistic electrons in a medium" by S Schneider and R Spitzer (*Nature,* **250,** 643, 1974) the following corrections should be made In equation (2) the factor $(\tau)\Theta(\gamma u\tau-\theta)$ should be replaced by $\theta(\tau)\theta(\gamma u\tau-\rho)$ The relationship $\varepsilon=n^2$, three lines above equation (2), should read $\varepsilon\neq n^2$

In the article "Nitrosoguanidine mutagenesis during nuclear and mitochondrial gene replication" by I W Dawes and B L A Carter (*Nature,* **250,** 709, 1974) Figs 1 and 4 were transposed The legends were given correctly

Reports and publications

Great Britain

It's Like This 500 Years of Pictures for Science (City of Nottingham Leisure Services—Nottingham Festival 1974) Pp 87 (Nottingham The Castle Museum and Art Gallery, 1974) 50p [257

University of Oxford Annual Report of the Curators of the Bodleian Library for 1972-1973 Pp 51 (Supplement No 5 to the *University Gazette*, June 1974) (Oxford The University, 1974) £1 [297

The Epistemological and Behavioural Basis of Culturalism a General Systems Approach (A Study of the Principles of Social Control in the Light of a General Model of Behaviour) By Edward Goldsmith Pp 27 (Wadebridge, Cornwall Wadebridge Ecological Centre, 73 Moleswirth Street, 1974) 80p [297

Medical Research Council Laboratory Animals Centre Manual Series, No 3 Survey of the Numbers and Types of Laboratory Animals Used in the United Kingdom in 1972 Pp 16 (Carshalton, Surrey MRC, Laboratory Animals Centre, 1974) [297

Philosophical Transactions of the Royal Society of London B Biological Sciences Vol 268, No 891 The Electron Microscopy and Composition of Biological Membranes and Envelopes A Discussion organized by R Markham, R W Horne and R Marian Hicks Pp 1–159 + plates 1–57 (London The Royal Society, 1974) [297

Medical Research Council Annual Report, April 1973–March 1974 Pp vi + 200 - 10 plates (London HMSO, 1974) 97p net [28

Philosophical Transactions of the Royal Society of London B Biological Sciences Vol 268, No 892 The Structure of the Stomatogastric Neuromuscular System in *Callinectes sapidus*, *Homarus americanus* and *Panulirus argus* (Decapoda Crustacea) By D M Maynard and M R. Dando Pp 161–220 £2 15 Vol 268, No 893 A Discussion on Human Adaptability in a Tropical Ecosystem—An I B P Human Biological Investigation of Two New Guinea Communities Organised by G A Harrison and R J Walsh Pp 221–400 £6 20 (London The Royal Society, 1974) [58

EMI Technology a Profile of Technological Capability Pp 30 (Hayes, Middx EMI, Ltd, 1974) [58

University of Oxford Ashmolean Museum—Report of the Visitors, 1972–73 Pp 61 (Supplement No 6 to the *University Gazette*) (Oxford The University, 1974) £1 [58

British Nuclear Fuels, Ltd Third Annual Report and Accounts, 1973–74 Pp 26 (Risley, Warrington British Nuclear Fuels, Ltd, 1974) [58

The Radiochemical Centre, Ltd, Amersham Third Annual Report for year ended March 31, 1974 Pp 16 (Amersham The Radiochemical Centre, 1974) [58

The Medical Research Council of Ireland Annual Report for the year ended December 31, 1973 Pp 89 (Dublin The Medical Research Council of Ireland, 1974) 25p [68

Grassland Research Institute Silver Jubilee Report, 1949/1974 Edited by C R W Spedding and R D Williams Pp 186 (Hurley, Maidenhead The Grassland Research Institute, 1974) £2 [98

Science Research Council Computational Chemistry and Physics (Science Board A joint report based on discussions of the Computational Chemistry and Physics Panels) Pp 42 (London Science Research Council, 1974) *gratis* [98

Other Countries

Water Quality Abstracts, Vol 2 Pp 482 (La Jolla, California Pollution Abstracts, Inc, 1974) [58

Plastic Containers for Pharmaceuticals—Testing and Control By Jack Cooper Pp 204 (Geneva World Health Organisation, London HMSO, 1974) Sw fr 32 [58

Smithsonian Contributions to Zoology No 154 *Ataenius*, *Aphotaenius*, and *Pseudataenius* of the United States and Canada (Coleoptera Scarabaeidae Aphodiinae) By Oscar L Cartwright Pp iv + 103 + 3 plates $1 85 No 174 Three New Crustaceans from La Media Luna, San Luis Potosi, Mexico By Alejandro Villalobos Figueroa and Horton H Hobbs, jun Pp iii + 18 30 cents (Washington, DC Smithsonian Institution Press, 1974 For sale by US Government Printing Office) [58

Berichte des Deutschen Wetterdienstes, Nr 133 Numerische Simulation Hochreichender Feuchtkonveltion auf der Basis der Ursprunglichen Eulerschen Gleichungen Von E Muller Pp 141 + 22 (Offenbach a M Selbstverlag des Deutschen Wetterdienstes, 1974) [58

Canada Department of Energy, Mines and Resources Geological Survey of Canada Catalogue of Type Invertebrate Fossils of the Geological Survey of Canada, Vol 5 Pp 396 $10 Bulletin 244 Middle Ordovician Ostracoda from Southwestern District of Mackenzie By M J Copeland Pp 55 (9 plates) $5 Paper 73–13 Rocks and Minerals for the Collector Cobalt-Belleterra-Timmins, Ontario and Quebec By Ann P Sabina Pp ix + 199 $3 Paper 73–30 Rocks and Minerals for the Collector Kirkland Lake-Noranda-Val d'Or, Ontario and Quebec By Ann P Sabina Pp viii + 162 $3 Paper 74–1, Part B Report of Activities, Part B, November 1973 to March 1974 Pp 297 $5 Paper 74–12 Tips on Organising Arctic Geological Field Work By J Wm Kerr p 12 $2 (Ottawa Information Canada, 1974) [58

Sveriges Geologiska Undersokning Avhandlingar och Uppsatser, Ser C, Nr 681 Isavsmaltningens Forlopp i Jamtlands Wan (Deglaciation of the County of Jamtlan, Central Sweden) By Jan Lundqvist p 187 (Stockholm Sveriges Geologiska Undersokning, 1973) [68

Indian Forest Records, New Series Entomology Vol 11, No 2 Descriptions and Records of some Indian Psyllidae (Homoptera) By R N Mathur Pp 59–87 Rs 160, 19p, $0 58 Forest Pathology Vol 2, No 11 Cultural Diagnosis of Indian Polyporaceae 3 Genera *Daedalea*, *Favolus*, *Ganoderma*, *Hexagonia*, *Irpex*, *Lenzites*, *Merulius* and *Poria* By Manjusri Sen Pp 277–304 + 11 plates Rs 4 20, 50p, $1 52 (Delhi Manager of Publications 1973) [68

United States Department of the Interior Geological Survey Bulletin 1394-D Concepcion and Palma Escrita Formations, Western Puerto Rico By David H McIntyre Pp iii + 9 30 cents Water-Supply Paper 2098 Quality of Surface Waters of the United States, 1968 Parts 9 and 10 Colorado River Basin and The Great Basin Pp ix + 296 $2 35 Water-Supply Paper 2100 Quality of Surface Waters of the United States, 1968 Parts 12–16 North Pacific Slope Basins, Alaska, Hawaii, and other Pacific Areas Pp xii + 464 $3 05 (Washington, DC Government Printing Office, 1973 and 1974) [68

Commissariat a l'Energie Atomique, Paris Cedex Rapport Annuel 1973 Pp 95 Activites Scientifiques et Techniques 1973 Pp 225 (Paris Cedex Commissariat a l'Energie Atomique, 1974) [68

World Health Organisation Technical Report Series, No 543 Food-Borne Disease—Methods of Sampling and Examination in Surveillance Programmes Report of a WHO Study Group Pp 50 (Geneva WHO, London HMSO, 1974) Sw fr 5 [78

nature
Volume 251 October 18, 1974

Investigating the paranormal

We publish this week a paper by Drs R. Targ and H. Puthoff (page 602) which is bound to create something of a stir in the scientific community. The claim is made that information can be transferred by some channel whose characteristics appear to fall "outside the range of known perceptual modalities". Or, more bluntly, some people can read thoughts or see things remotely.

Such a claim is, of course, bound to be greeted with a preconditioned reaction amongst many scientists. To some it simply confirms what they have always known or believed. To others it is beyond the laws of science and therefore necessarily unacceptable. But to a few—though perhaps to more than is realised—the questions are still unanswered, and any evidence of high quality is worth a critical examination.

The issue, then, is whether the evidence is of sufficient quality to be taken seriously. In trying to answer this, we have been fortunate in having the help of three independent referees who have done their utmost to see the paper as a potentially important scientific communication and not as a challenge to or confirmation of prejudices. We thank them for the considerable effort they have put in to helping us, and we also thank Dr Christopher Evans of the National Physical Laboratory whose continued advice on the subject is reflected in the content of this leading article.

A general indication of the referees' comments may be helpful to readers in reaching their own assessment of the paper. Of the three, one believed we should not publish, one did not feel strongly either way and the third was guardedly in favour of publication. We first summarise the arguments against the paper.

(1) There was agreement that the paper was weak in design and presentation, to the extent that details given as to the precise way in which the experiment was carried out were disconcertingly vague. The referees felt that insufficient account had been taken of the established methodology of experimental psychology and that in the form originally submitted the paper would be unlikely to be accepted for publication in a psychological journal on these grounds alone. Two referees also felt that the authors had not taken into account the lessons learnt in the past by parapsychologists researching this tricky and complicated area.

(2) The three referees were particularly critical of the method of target selection used, pointing out that the choice of a target by "opening a dictionary at random" is a naive, vague and unnecessarily controversial approach to randomisation. Parapsychologists have long rejected such methods of target selection and, as one referee put it, weaknesses of this kind reveal "a lack of skill in their experiments, which might have caused them to make some other mistake which is less evident from their writing".

(3) All the referees felt that the details given of various safeguards and precautions introduced against the possibility of conscious or unconscious fraud on the part of one or other of the subjects were "uncomfortably vague" (to use one phrase). This in itself might be sufficient to raise doubt that the experiments have demonstrated the existence of a new channel of communication which does not involve the use of the senses.

(4) Two of the referees felt that it was a pity that the paper, instead of concentrating in detail and with meticulous care on one particular approach to extra-sensory phenomena, produced a mixture of different experiments, using different subjects in unconnected circumstances and with only a tenuous overall theme. At the best these were more "a series of pilot studies … than a report of a completed experiment".

On their own these highly critical comments could be grounds for rejection of the paper, but it was felt that other points needed to be taken into account before a final decision could be made.

(1) Despite its shortcomings, the paper is presented as a scientific document by two qualified scientists, writing from a major research establishment apparently with the unqualified backing of the research institute itself.

(2) The authors have clearly attempted to investigate under laboratory conditions phenomena which, while highly implausible to many scientists, would nevertheless seem to be worthy of investigation even if, in the final analysis, negative findings are revealed. If scientists dispute and debate the reality of extra-sensory perception, then the subject is clearly a matter for scientific study and reportage.

(3) Very considerable advance publicity—it is fair to say not generated by the authors or their institute—has preceded the presentation of this report. As a result many scientists and very large numbers of non-scientists believe, as the result of anecdote and hearsay, that the Stanford Research Institute (SRI) was engaged in a major research programme into parapsychological matters and had even been the scene of a remarkable breakthrough in this field. The publication of this paper, with its muted claims, suggestions of a limited research programme and modest data, is, we believe, likely to put the whole matter in more reasonable perspective.

(4) The claims that have been made by, or on behalf of, one of the subjects, Mr Uri Geller, have been hailed publicly as indicating total acceptance by the SRI of allegedly sensational powers and may also perhaps now be seen in true perspective. It must be a matter of interest to scientists to note that, contrary to very widespread rumour, the paper does not present any evidence whatsoever for Geller's alleged abilities to bend metal rods by stroking them, influence magnets at a distance, make watches stop or start by some psychokinetic force and so on. The publication of the paper would be justified on the grounds of allowing scientists the opportunity to discriminate between the cautious, limited and still highly debatable experimental data, and extravagant rumour, fed in recent days by inaccurate attempts in some newspapers at precognition of the contents of the paper.

(5) Two of the referees also felt that the paper should be published because it would allow parapsychologists, and all other scientists interested in researching this arguable field, to gauge the quality of the Stanford research and assess how much it is contributing to parapsychology.

(6) *Nature,* although seen by some as one of the world's most respected journals cannot afford to live on respectability We believe that our readers expect us to be a home for the occasional 'high-risk' type of paper This is hardly to assert that we regularly fly in the face of referees' recommendations (we always consider the possibility of publishing, as in this case, a summary of their objections) It is to say that the unusual must now and then be allowed a toe-hold in the literature, sometimes to flourish, more often to be forgotten within a year or two

The critical comments above were sent to the authors who have modified their manuscript in response to them We have also corresponded informally with the authors on one or two issues such as whether the targets could have been forced by standard magical tricks, and are convinced that this is not the case As a result of these exchanges and the above considerations we have decided to publish in the belief that, however flawed the experimental procedure and however difficult the process of distilling the essence of a complex series of events into a scientific manuscript, it was on balance preferable to publish and maybe stimulate and advance the controversy rather than keep it out of circulation for a further period

Publishing in a scientific journal is not a process of receiving a seal of approval from the establishment, rather it is the serving of notice on the community that there is something worthy of their attention and scrutiny And this scrutiny is bound to take the form of a desire amongst some to repeat the experiments with even more caution To this end the *New Scientist* does a service by publishing this week the results of Dr Joe Hanlon's own investigations into a wide range of phenomena surrounding Mr Geller If the subject is to be investigated further—and no scientist is likely to accept more than that the SRI experiments provide a *prima facie* case for more investigations—the experimental technique will have to take account of Dr Hanlon's strictures, those of our own referees and those, doubtless, of others who will be looking for alternative explanations

Perhaps the most important issue raised by the circumstances surrounding the publication of this paper is whether science has yet developed the competence to confront claims of the paranormal Supposedly paranormal events frequently cannot be investigated in the calm, controlled and meticulous way that scientists are expected to work, and so there is always a danger that the investigator, swept up in the confusion that surrounds many experiments, abandons his initial intentions in order to go along with his subject's desires It may be that all experiments of this sort should be exactly prescribed beforehand by one group, done by another unassociated group and evaluated in terms of performance by the first group Only by increasing austerity of approach by scientists will there be any major progress in this field

For those in peril on the factory floor

In this article Peter J Smith argues that a greater commitment (in deed as well as word) to community science by the Scientific Establishment might help the world of science regain some of the public respect it has lost

THE question of who speaks, or should speak, on behalf of the scientific community has been debated on many occasions, most often without result On the face of it, such lack of resolution is hardly unexpected, for scientists and scientific institutions are not noted for their ready ability to achieve consensus Yet there is no doubt that they can put up a pretty collective front when they feel so moved The one famous occasion on which a near consensus was reached was when the scientific community saw itself put at risk financially by the Rothschild proposals Then individuals and institutions miraculously found a common cause of self-preservation

But when it comes to the defence of less privileged groups it is quite a different story, the voice of the British scientific community is seldom to be heard, whether taking a moral stance, exerting humanitarian pressure, supplying expertise or even simply providing information A good case in point is provided by a new *Socialist Worker* pamphlet entitled *Asbestos The Dust that Kills in the Name of Profit* As the title hints, the object of *Socialist Worker* is nothing less than the complete overthrow of the capitalist system, and one of the ways of achieving this aim, it seems, is to give strident publicity to defects in the capitalist-industrial system Fortunately, one can easily avoid a sharp turn to the left and still admit that what some British workers have been subjected to in the name of asbestos production is beyond the limit of acceptability in a humanitarian society

For what clearly emerges from the rhetoric of the pamphlet in question is a picture of men and women reacting in some bewilderment to the long-term ill effects of a technological activity The chief consequence is, of course, asbestosis—a killing disease acquired by breathing in asbestos fibres The bulk of the pamphlet is devoted to case histories of men to whom asbestosis has come as a shock after a decade or so in the industry But more instructively, there is also a short account of the fight for safety put up by a small group of the 7/162 Glasgow insulation workers' branch of the Transport and General Workers Union against the obstruction of the asbestos companies, the indifference of politicians, the weakness of the Factory Inspectorate, the silence of much of the press, the impotence of health authorities, the equivocal official stance of unions in general, and, last but not least, apathy among many of the asbestos workers themselves

And there is certainly something to fight about According to Patrick Kinnersly (*The Hazards of Work How to Fight Them,* Pluto Press, 1973), asbestosis is taking an increasing toll 64 are known to have died in 1965, 107 in 1970 and 113 in 1971 The number of new cases diagnosed rose from 82 in 1965 to 153 in 1970 Moreover, asbestosis is only one of the asbestos-induced diseases Lung cancer appears to require a smaller exposure to asbestos There is also another form of cancer known as mesothelioma which involves growths in the linings of the lungs and stomach Almost all mesotheliomas are caused by asbestos, but no one knows how many workers in Britain are killed by them, partly because they take so long to develop and partly because they are not always identified The TUC Centenary Institute of Occupational Health has suggested that, 30 years after first exposure, about one in 200 will be found to have died of mesothelioma, but Dr Irving J Selikoff of Mount Sinai Hospital in New York is apparently more pessimistic He has recently been quoted as saying that, for every 100,000 workers entering the asbestos industry under the safety standards obtaining in the United States as recently as 1971, he would expect 20,000 to die of lung cancer, 7,000 of mesothelioma and 7,000 of other cancers and asbestosis

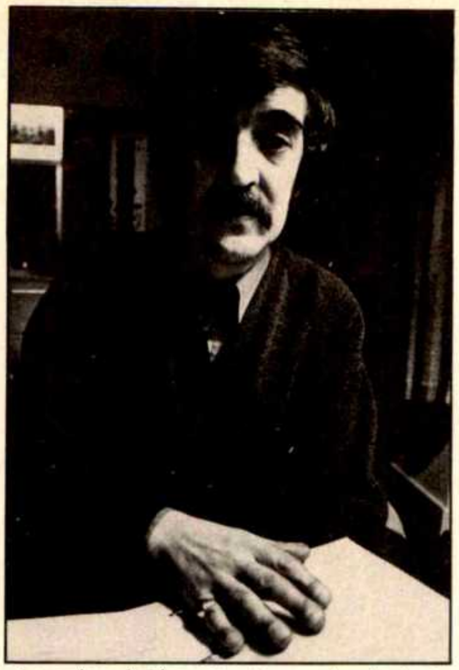
The point of drawing attention to the asbestos problem is not to elevate it into some sort of special case; any one of a hundred examples could just as easily be used for illustration. Nor is it to argue that the whole asbestos industry should be closed down forthwith. Such a course of action would be inconceivable in the face of the fact that asbestos currently finds some 3,000 uses in almost every sphere of material living (a testimony to its unique combination of fibrosity and imperviousness to heat). Asbestos products (and thousands of other equally or more dangerous substances) are here to stay.

But under what conditions are they to stay? And who is to influence those conditions? The most striking thing to emerge from the story of the asbestos workers of Glasgow and elsewhere is that no organisation remotely connected with the world of established science has played any part in it. The British Association has been silent. The Science and Natural Environment Research Councils have been silent. The Royal Institution has been silent. The University Grants Committee has been silent. The Association of University Teachers has been silent. In this and many other comparable cases a few individual scientists and science journalists have spoken and acted; but what has been completely lacking is the evidence of any sense of collective responsibility on behalf of the whole, or even a significant part, of the British scientific community.

It is true of course, that the Medical Research Council and the recipients of their grants are responsible for a great deal of basic biological and medical research into this and other industrial diseases; and my remarks should not be construed as criticism of that organisation, the individuals associated with it or the valuable work they do. The point I make is much more general—that even when the adverse side-effects of science and technology directly involve health (and they do not always do so), their mitigation requires much more than fundamental research into the nature of the disease itself.

Here, with particular reference to the asbestos case but with general applicability to many other situations, are some specific things which the scientific community could (and should) have done and could still do.

- Admit moral responsibility for taking a clear lead in seeing that the ill effects of science and technology are eliminated or at least mitigated. As Dr. Bernard Dixon (*What is Science for?*, Collins, 1973) has said: "When an unexpected, unforeseen calamity does happen, the scientist is more, not less or equally, responsible compared with others in doing all he can to fight against it". In practical terms, taking a moral lead would probably first mean overseeing the organisation of a suitable team to map out the full extent of the problem and identify gaps in existing knowledge. Between them, the organisations mentioned above encompass the elite (albeit often self-elected), if not most participants, of British science, and form a body with an almost infinite variety of expertise and an immense potential for influencing public and government opinion. That these powers are so often allowed to lie dormant is, to say the least, astonishing.



A worker displays symptoms of asbestosis

- Ensure that the outstanding questions and problems identified by the investigating team are followed through. In almost all cases it is probable that more research is needed, in which case the distinguished bodies of science should regard it as their duty to create the necessary political, financial and organisational climate in which such research can be carried out. Is monitoring equipment highly enough developed? Are safety levels adequate and are they formulated in the right way? To what extent is the general public, as well as factory workers, at risk? These and many other questions covering a variety of disciplines can be investigated but they require effort. Again, the principal scientific organisations have it in their power to encourage the mobilisation of scientists for this purpose. Will they use this influence?
- Exert pressure to ensure that the high principles so proudly proclaimed by the scientific community are actually put into effect. For example, it is quite plain that one of the greatest difficulties encountered by industrial workers and others exposed to risk is that of securing the scientific data (for example, from monitoring) from which a proper assessment risk may be made. Up to July of this year, Factory Inspectors divulging such information to employees or others without the employer's permission were actually subject to imprisonment. The Health and Safety at Work Act 1974 has mitigated these harsh conditions, although as the Trade Union Research Unit at Oxford has pointed out (*Health and Safety at Work Legislation: A Critical Study*, 1974), disclosure of information is still left largely to the Factory Inspector's discretion. It is difficult to understand how a community which puts such a high value on the freedom of scientific information can reasonably continue to allow the principle to be treated with contempt. Is there any respectable reason why human health should be less privileged in this respect than the activity of pure science? Of course, employers frequently attempt to justify their refusal to disclose vital information on the grounds of commercial secrecy—a ploy which is sometimes legitimate, sometimes not. The scientific community has a unique role to play here, for a group of independent scientific experts of integrity is the only body able to make a proper assessment of what is truly confidential and what is unreasonably claimed as such.

Why has the scientific community as a whole failed in this respect? The reason seems to be that, in the past, scientists have found it adequate to justify widespread public support of science on the grounds of the supposed cultural value of the activity and of its supposed role as the progenitor of technology. But what they have failed to grasp is that the first of these grounds was never very convincing to the wider world (at least as justification for a high level of public support) and that the second is rapidly losing its credibility.

In fact, science has a third role, which is neither "science for its own sake" nor science conceived as a means to a materialistic end. This is science for human welfare—call it "community science" if you like. It is practised in a few university departments; it is practised with great effect by, for example, the staff of the Greater London Council's Scientific Adviser. It is regarded as second rate by most university scientists, as a threat by industry, and with indifference by our major scientific institutions. But it is just possible that a greater commitment to it by the scientific community might go some way towards regaining for science the public respect (not to mention the public money) it has so patently lost in recent years. ☐

international news

Frameworks for energy in the US...

by Colin Norman, Washington

On the eve of its recess for the November elections, the US Congress approved what could turn out to be the most significant piece of energy-related legislation to reach the lawbooks since the oil crisis began to bite.

Minutes before President Ford appeared before a joint session of Congress to deliver his economic pep talk, a House-Senate Conference committee reached agreement on a bill which will abolish the Atomic Energy Commission and replace it with a Nuclear Regulatory Commission (NRC) and an independent Energy Research and Development Administration (ERDA). Two days later, the bill was sitting on Ford's desk awaiting his signature. Until last week, the bill had been making glacial passage through the Congressional mill, and there were fears that no agreement would be reached on the measure before the end of the session.

The importance of the legislation is that it consolidates energy research and development programmes which are now spread out over a host of federal departments and agencies into a single agency. Furthermore, it removes a long standing complaint that the Atomic Energy Commission suffers from a conflict of interest by both promoting and regulating nuclear energy, because it splits those functions into separate agencies. Furthermore, the bill considerably elevates the status of research programmes concerned with nuclear safety and the safeguarding of nuclear materials.

The bill, which was originally proposed by former President Nixon's Administration, sets up the NRC to take over virtually all of the regulatory functions that are now performed by the Atomic Energy Commission, but it also completely alters the present bureaucratic structure. The NRC will consist of three co-equal offices, each of which will report to a 5-member commission. The Office of Nuclear Reactor Regulation will be concerned with licensing nuclear reactors. The Office of Material Safety and Safeguards will license reprocessing facilities and facilities associated with transporting nuclear materials, and it will also make sure that nuclear materials are adequately safeguarded.

The third, the Office of Nuclear Regulatory Research, will perform backup research for evaluating licence applications to ensure that safety and safeguards criteria are met.

As for ERDA, it will take over virtually all the energy research and development activities performed by the federal government, using the laboratories of the Atomic Energy Commission as the base of its operations. It will be split into six divisions, each of which will be headed by an Assistant Administrator, concerned with fossil fuels; nuclear energy; environmental safety; conservation, solar, geothermal, and advanced energy systems; and nuclear security.

ERDA will carry out all the energy programmes related to Project Independence—the much vaunted drive to make the United States self-sufficient in energy supplies—and it will be armed with a huge budget, amounting at present to about $2,000 million a year. Nuclear energy will soak up nearly half of the ERDA funds, with the breeder reactor getting the biggest single slice. But it will also take over such programmes as the solar energy research effort which was recently launched by Congress (see *Nature*, 251, 368, 1974), as well as Congressionally inspired research and development programs concerned with geothermal energy and other longer-term energy options. □

... and in Holland

from Arie de Kool, Amsterdam

The Netherlands are going nuclear, but slowly, and with a maximum of government control. In fact, the government will increase its influence in the whole of the energy field considerably—if possible, together with other European countries, if not, alone.

Its proposals are set out in a long-awaited White Book on Energy. Several of these are concerned with getting the government a measure of control in the privately owned energy supply sector. As far as nuclear power is concerned, the government proposes to establish a monopoly for the exploitation of nuclear power stations and promises to undertake studies on overall nuclear safety within two years. It plans to build three 1,000 MW nuclear power stations, to be on stream by 1985 or when the establishment of the government nuclear monopoly and the safety studies permit.

The government also wants to negotiate a say in the managerial conditions of energy supply corporations, and by bringing the planning of all types of power stations under government responsibility, ensure that electrical power supply is discussed in parliament. To stimulate the use of coal for electricity generation the government will stipulate that all new power plants shall be fitted for oil and coal firing. The Dutch will also continue to participate in the German-Belgian-Dutch prototype sodium-cooled fast breeder reactor at Kalkar in Germany, although the government is strongly considering pulling out of the next phase, the construction of a 1,000 MW demonstration reactor.

Looking outwards, the government plans to take a 40% share in all natural gas and oil exploitations on the Dutch part of the continental shelf, with the condition that the government share in the profit may rise as high as 80% if conditions allow "without impeding the attractiveness to the oil companies". The Dutch government also plans to build up a "strategic reserve" of natural gas by stimulating exploration and exploitation of smaller fields in order to have the major source at Slochteren available in case of another oil crisis.

It is no secret, that the cabinet has been highly divided, especially over the use of nuclear energy. A couple of days before the White Book was officially released, it leaked out that the government proposed a considerable delay in the construction of nuclear power plants. This was the interpretation the 'progressive' members of the cabinet were giving (and were supposed to give) to the delay that is bound to result from the establishment of the government monopoly and the requirements of an overall safety report.

However, Dr Lubbers, Minister of Economic Affairs, maintained at a press conference, that the three plants should be ready by 1985. The safety reports might lead to a change in reactor type, a change of site, even to a postponement of the start of the first reactor, but not to the goal of 3,500 MW of nuclear energy in 1985.

Naturally, the opponents of nuclear energy are not very happy with the White Book. Some 10,000 people biked and bussed to Kalkar in protest, three days after the White Book was published. □

The chemistry of profits

from Nechemia Meyers, Rehovot

DETAILED plans are now being made for an investment of some £240 million in Israel's chemical industry. Roughly half this sum will be used to promote the exploitation of inorganic Negev chemicals, with the other half being used to expand the Haifa Bay complex of petrochemical firms.

The investment, enormous in Israeli terms, is a natural outcome of the chemical industry's growing success, both in supplying vital products to the home market and in the export sphere. Overseas sales of Israeli chemicals already account for 20% of her industrial exports (excluding diamonds), and there is no apparent limit to potential customers.

The 393-square-mile Dead Sea, most saline of the world's lakes, has long been the major focus of Israel's chemical industry in the 20th century. Plans for exploiting its potash and other mineral resources were drawn up in 1911 by Moshe Novomeysky (an immigrant from Siberia) and the first plant, run by Novomeysky, began operation in 1931. For many years, however, the Dead Sea Works were usually in the red, causing cynics to suggest that "the only thing that can sink in the Dead Sea is money".

It sinks no longer. On the contrary, Dead Sea potash, bromine and magnesium oxide—extracted from a 50-square-mile area of evaporation pans at the southern end of the saline lake whose waters initially contain only 1% (by weight) of potassium chloride—are today sold at a handsome profit to customers in Europe, Africa and the Far East.

Other Negev enterprises are also profitable, in large measure because of rising prices. Israel's phosphate mines—part of the Mediterranean phosphate belt that stretches from Morocco in the west to Jordan in the east and Turkey in the north—are a striking case in point.

In 1971 Government experts, dismayed by 20 years of losses, suggested that they be closed down. Fortunately they were not, and as a result profits are now rolling in to the State Treasury. Ironically, they largely reflect Arab Morocoo's successful exploitation of the world-wide fertiliser shortage to push up phosphate prices from $11 to $60 a ton in just one year.

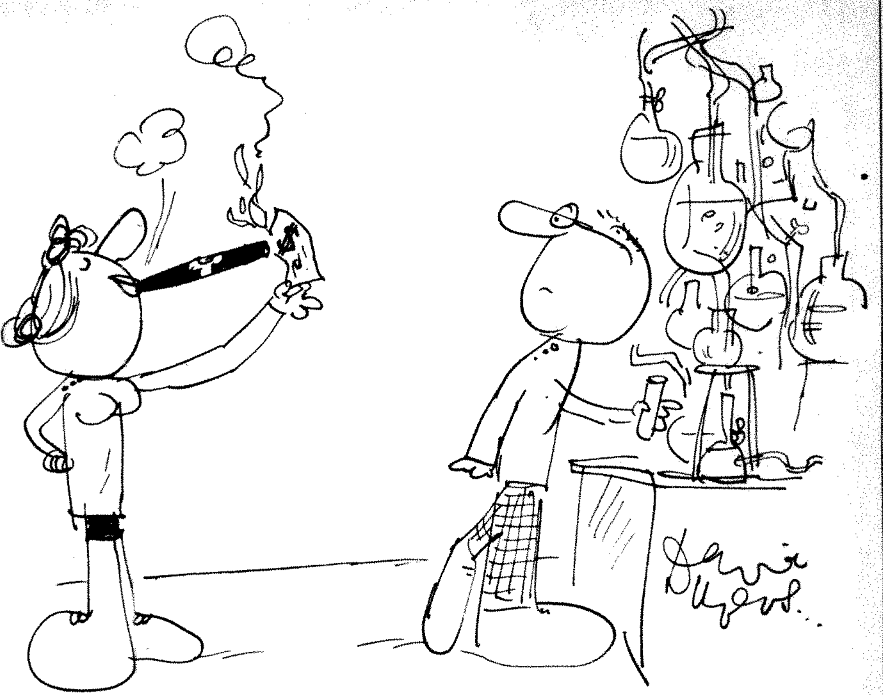
Copper prices are now lower than they were at their peak, but they are still high enough to ensure the profitability of the modern copper mines at Timna (which are very near the Chalcolithic ones). However, the copper-bearing strata that were worked in ancient times are not the same as those mined today. Formerly copper nodules associated with fossilised trees within the Middle White Sandstone were exploited. This layer appears near Timna about 330 feet above the copper-bearing Cambrian rocks that are now worked (by both underground and open-cast mining).

The only major failure in this sphere has been with a controversial plant, near the Judean Desert town of Arad, for the manufacture of phosphoric acid by means of hydrochloric acid produced from residual Dead Sea brines. An American firm whose fluidised refining process was used in the plant, the Madera Company, is being sued in an attempt to recover at least part of the £25 million loss, and alternative operating methods are now being studied.

When the current expansion plan is completed, potash production will rise from one million to 1.5 million tons a year, phosphate production from one million to 2.8 million tons a year and bromine production from 20,000 to 50,000 tons annually. Profits from the mining and processing of Negev chemicals, this year expected to total £15 million, will presumably also rise in at least the same measure.

The scheduled £240 million investment in the petrochemical industry will meet local needs for petrochemicals (including raw materials for plastics) and also give a boost to exports. Items whose production is to rise include polyethylene, styrene, polystyrene, phenolics and PVC. In addition, much larger quantities of potassium nitrate — a highly regarded, Israeli-developed chemical fertilizer—will be produced in the Haifa area.

While the cost of the oil used as a base for these petrochemical products has obviously risen steeply, Israel is no worse off in this respect than her competitors, and looks ahead to profits from petrochemicals in addition to those she is already earning from inorganic chemicals. □



WOMEN who graduated from American universities this year with a degree in chemistry or chemical engineering commanded higher salaries than their male counterparts, according to a study carried out by the American Chemical Society. It is the first time since the society began keeping track of such matters that women graduates have earned more than men.

The ACS survey showed that, on average, the starting salary for women chemists this year was 5% higher than that for men, whereas in 1964, newly graduated women chemists could expect to earn only 68% of the starting salary for men. While this seems to indicate that employers are attempting to recruit more women, the survey also turned up evidence that the battle for equal employment opportunities still has some way to go. For one thing, women and minority groups had a higher unemployment rate than the average for all ACS members. The overall rate of 1.4% was exceeded by American Indians (6.3%), Orientals (3.1%), Spanish surnamed and black chemists (1.5%), and women (3.5%). But at least that's an improvement on previous years, when the unemployment rate among women chemists was typically running at about three times that for men.

ICI puts money on genetic engineering

by Miranda Robertson

THE news that Britain's Imperial Chemical Industries Ltd (ICI) is to invest £40,000 over the next three years in research on genetic engineering initiated by University of Edinburgh biologists seems to bring the more optimistic speculations on the potential benefits of these controversial techniques a step closer to reality. One of the most widely cited possibilities has been the production of insulin from a mammalian gene inserted into a bacterial phage or plasmid. Yet projects aimed at this kind of application, including the Edinburgh-ICI collaboration, are still very much at the research, and short of the development, stage.

The outstanding problem takes the form of a fundamental question in molecular biology: will the synthetic machinery of a bacterium lend itself to the production of a protein specified by a mammalian (or any other eukaryotic) gene? Morrow and his colleagues in the United States have succeeded in demonstrating that bacteria can transcribe eukaryotic DNA into RNA (*Proc. natn. Acad. Sci.*, **71**, 1743; 1974); but whether they can achieve the next step—the translation of the RNA into protein—is still not known. Furthermore, this is much the trickier step of the two, for while the genetic code is universal, the software for translating it into protein is not. Optimists believe that the first eukaryotic protein will be emerging from bacterial cells within the next year or two. Equally distinguished sceptics foresee that the highly specific molecular recognition and control systems governing protein synthesis will present serious problems.

Dr Kenneth Murray, whose work with his wife Noreen on phage lambda (*Nature*, **251**, 476; 1974) led to the ICI project, suspects that bacteria can be made to translate mRNA specified by eukaryotic genes, though perhaps rather inefficiently. One way in which he can conceive of overcoming specificity problems is by reducing the inserted DNA to the bare structural gene, as free as possible from regulatory sequences that would be unrecognisable to a bacterial synthetic system.

That raises the further technical problem of accurate excision of single genes from the donor DNA. The current burst of genetic tailoring took off from the isolation in 1972 of the bacterial restriction enzyme *Eco*RI, whose peculiar properties make it particularly suitable for the cleavage and reconstitution of heterologous DNA. But the very property of specific cleavage that makes the enzyme so useful also makes it inflexible: it will only cut a molecule where it wants to, and not necessarily where you want it to. Consequently one of the first requirements will be a range of enzymes with different specific cleavage sites. Researchers at Edinburgh and elsewhere are already at work on the accumulation of such a versatile set of molecular scalpels, but no-one is yet ready even to think in concrete terms about operating on such exotica as insulin genes.

With the recent emphasis on the possible hazards of genetic engineering research, safety has become a prominent issue. In fact, the experiments on phage lambda fall outside the forbidden categories I and II of the US National Academy of Sciences' cautionary statement, into the more amorphous category of experiments which "should not be undertaken lightly". One of the principal points which Murray has stressed in connection with the phage lambda system is its relative intrinsic safety. It does not carry drug resistance factors and is therefore susceptible to control, and has only been used so far with strains of *E. coli* to be found in the human gut. If the system comes into use on a commercial scale, Dr Murray is willing to envisage the development of bacterial protein factories custom-built to be incapable of infecting a human host.

THE bacterial engineering argument so far has been given most prominence in the United States and Britain, and it is likely that these two countries will be the first to formulate a set of guidelines, either as legal constraints or as informal codes of practice.

But the problem is worldwide, and the International Association of Microbiological Societies has recently set up an *ad hoc* committee to advise it on the hazards inherent in recently developed techniques of genetic engineering.

This committee will not attempt to duplicate the activities of either Professor Paul Berg and his committee in the United States or Lord Ashby's Working Party in the United Kingdom, according to the chairman, Professor S. W. Glover of the University of Newcastle-upon-Tyne. Rather it hopes to evaluate any recommendations made by these committees or at the international meeting planned to take place in California next February, and if necessary, to arrive at a set of constraints or guidelines which it can recommend to the IAMS for worldwide communication through the network of national societies affiliated to the IAMS.

Apart from Professor Glover, the committee consists of Dr E. Wollman (France), Dr A. Demain (US) and Professor G. Terui (Japan).

In the meantime, precautions at Edinburgh include scrupulous sterilisation procedures. So far, the Murrays themselves have been the only people to have handled doctored phage particles. Not that they really believe that phage presents a serious threat to health; with the courage of their conviction, each Murray swallowed an experimental dose of 10^9 labelled phage—without either immediate ill effects, or traces of surviving organisms in the gut flora thereafter.

The injection of ICI funds will now, of course, make it possible to adapt a laboratory designed to contain pathogens, while at the same time the expansion of the project will mean the involvement of more personnel. Proper training of laboratory personnel will be emphasised since structural safeguards such as air-locks and protective hoods—both on the agenda—offer no protection from human carelessness.

The obvious advantage of an academic-industrial collaboration such as that between Edinburgh University and ICI is the pooling of intellectual resources not normally available to industry and practical resources not normally available to universities. There is one university department, however, which is uniquely placed from both points of view. The Department of Biochemistry at Imperial College London possesses a pilot plant for growing up to 60 kg of bacteria, as well as facilities for purifying large quantities of enzymes, and is now under the chairmanship of Professor Brian Hartley. Hartley and his collaborators have been working for the past few years on enzyme adaptations in bacteria (see for example *Nature*, **251**, 200; 1974). The most recent development in this work has been the use of genetic engineering techniques to transfer the genes specifying the enzymes between bacterial species. Hartley's plans for the biochemistry department include the recruitment of young molecular geneticists with a view to developing and exploiting these sophisticated techniques in the production of commercial quantities of enzymes tailor-made to the purposes of medicine and industry.

His approach differs from that of the Edinburgh team in a number of ways. For one thing, their project is based principally on the use of phage lambda as the vehicle for the transplanted genes, whereas Hartley's will involve both phage (where that seems advantageous) and bacterial plasmids. (This will bring the Imperial College project into NAS category I, so that out of respect for the moratorium it will have to be deferred, though probably not for long.) But there are also

Business report

by Roger Woodham

FACED with two proposals for dealing with their company's liquidity crisis, employees of George Kent Ltd have voted in favour of an arrangement with the Swiss company Brown Boveri which would give Kent more cash to the tune of £6 million but leave effective control of the company in the United Kingdom The name Kent on scientific instruments is well known in its own right in research laboratories, as is that of a company which it owns—Cambridge Scientific Instruments

The result of this exercise in industrial democracy flew in the face of Mr Anthony Benn, Secretary of State for Industry, who preferred an arrangement whereby George Kent would be taken over by GEC, Sir Arnold Weinstock's company But Mr Benn was also keen that Kent's employees should have a chance to make their views known and he now has to decide, in the light of the ballot, how to use the 24% of the Kent shares which the government owns when it comes to a vote among shareholders If he decides to go along with the Brown Boveri plan it seems almost certain to be the one that will win the day

Brown Boveri originally proposed that it should buy 53% of the Kent shares but it has now decided to be content with less than 50%, thus assuaging fears that control of George Kent would be lost to a foreign company It also envisages Kent's scientific and medical instruments business being set up as a separate company owned by the mooted Brown Boveri Kent Ltd, and this is where some Kent employees part company with the Brown Boveri plans The 600 employees of Cambridge Scientific Instruments voted in favour of the GEC plan to a man because they regard an independent instrument company as a doubtful starter because of its small size Over George Kent as a whole, however, 75% of the 7,300 employees said yes to Brown Boveri

This desire to run into the arms of of a Swiss-based multinational is almost certainly based on fears that 'rationalisation' after a takeover by GEC would threaten job security By contrast there is little overlap between the activities of Kent and Brown Boveri, and the latter sees Kent as a useful marketing base in the United Kingdom Indeed the prospect of access to France, Germany and Switzerland, where Brown Boveri sells some £780 million worth of equipment and where neither Kent nor GEC is particularly strong, is an added attraction from Kent's point of view

major differences in both methods and aims With his background in enzyme evolution and bacterial adaptation, Hartley expects to rely very much more heavily than the Murrays on the adaptive potential of the bacteria themselves This might be used, for example, to produce enzymes stable enough to survive long periods of storage at room temperature The first step would be to introduce the gene into *Bacillus stearothermophilus,* which thrives at high temperatures If an environment could then be devised in which the bacterium's survival would be greatly enhanced by the production of large quantities of the new enzyme, the bacterium would be under considerable selective pressure not only to make the enzyme, but to evolve a structure for it that would be stable at high temperatures

In a collaborative effort with the Microbial Research Establishment at Porton, Hartley has already achieved the purification to homogeneity of 20 enzymes from thermophilic bacteria, in quantities of 50 kg The facilities are to be moved to Imperial College in January 1975, and the intention is eventually to produce enzymes for sale to industry There is also the possibility of selling enzymes for research to other laboratories Imperial College has at its disposal a potential wealth of restriction enzymes in the vast range of organisms grown there for antibiotic research Isolation and purification of these enzymes will be an important part of the research programme

The problem of maximising bacterial production of a particular enzyme for either commercial or research purposes may be another case in which the answer lies in the versatility of the bacteria themselves In the case of phage lambda, there is a well known promotor system which it may be possible to commandeer This enzyme system, together with the product of another phage gene, can override the controls which normally serve to prevent or limit the transcription of a given gene Up to half the cell's synthetic capacity may in consequence be recruited to the production of a single type of protein molecule In bacteria, however, it may actually be possible to increase the number of genes from which transcription of a given mRNA taken place Hartley and his colleagues have recently demonstrated (*Nature,* **251,** 200, 1974) that bacteria often respond to metabolic demands for large quantities of a particular enzyme by duplication of the genes which code for it Again, of course, this technique would involve manipulation of the environment and not the bacterium

Hartley draws a categorical distinction between the prospect of getting bacteria to mass-produce enzymes to human specifications (science), and that of inducing them to make mammalian hormones such as insulin (science fiction) This is partly because of his own emphasis on bacterial evolutionary potential in the implementation of human designs While it may not be too difficult to devise environmental exigencies that would force a bacterium into an unnatural need for an *outré* enzyme, it is extremely difficult to imagine how one might provide it with an incentive to produce insulin—although with elaborate genetic manipulation it may be possible Hartley is not, however, among those who think translation will be a major problem, though he concedes it is the rate-limiting step But in view of the extensive use already made in industry of microbial enzymes, not to mention long-term plans for solving the global food problem with microbial proteins, bacterial enzyme production may prove quite enough to be getting on with □

Little Red greenery book

from our Soviet Correspondent

THE all-Union Botanical Society of the USSR has completed compilation of its "Red Book" of flora, listing some 20,000 forms in need of governmental protection, some 600 of which are in immediate danger of extinction

In commenting on the report, *Pravda* predictably pays prime attention to those species which provide "irreplaceable" raw material, such as the Siberian and Korean cedars, and the Caucasian ironwood, and notes that such valuable species as the waterfall poplar, Vavilov's almond, and the Kolyma currant are apparently, already, irretrievably lost

Nevertheless, the red book also includes plants which have so far found no economic use, including certain weeds "against which mankind has waged an agelong struggle" This would appear, at first glance, contrary to Soviet utilitarian theories, but, the commentator explains, the "dialectics of 'harm' and 'use' is in this case complicated and contradictory", and quotes the classic case of penicillin in order to justify the presence of the weeds □

Musical chairs on Capitol Hill

by Colin Norman, Washington

FOR six days early in October the US House of Representatives put on a display of squabbling and infighting which would almost have done justice to Gladstone's Parliament during the stormy days of Irish 'reform' One venerable Representative announced at one point that she would "fight to the death" to get her way, while another remarked that the House had lost its collective sanity In the end, however, there were no open fisticuffs on the floor, as there were in Gladstone's day, and the whole affair got scant attention from the general press But for science and technology, the titanic struggle on Capitol Hill holds some important implications

The basis of the squabbling was a bold attempt to reform the House's antiquated committee structure—not exactly a heart-stopping issue, which is probably why it did not grab too many headlines—and the outcome can best be described as a standoff between the reformers and those who were hoping to preserve the *status quo* But it will, at least, change the way in which the House handles a good deal of legislation involving research and development

In short, the committee on Science and Astronautics, which has never exactly been a giant on the Congressional landscape and whose influence has been shrinking recently along with the decline in the space programme, is set to pick up some important new responsibility

In addition to its present authority, which is essentially limited to the programmes of NASA, the National Science Foundation, the National Bureau of Standards and general science policy deliberations, the committee will pick up jurisdiction over all non-nuclear energy research and development, environmental research and development, civil aviation research and development and the Weather Service In line with its new roles, the committee also gets a new title—the Committee on Science and Technology

Certainly, it can be argued that the committee still cannot be called the focal point for scientific matters in the House since the really big spenders remain outside its jurisdictional patch Defence research and development stays under the jurisdiction of the Armed Services Committee, for example, the Commerce Committee retains control over biomedical research, and the Joint Committee on Atomic Energy has lost none of its authority over nuclear matters But the changes could turn out to be significant, particularly in regard to energy research and development

First, by way of background, some remarks are in order on the functions of Congressional committees and the need for reform

Committees in the House resemble a collection of mediaeval fiefdoms presided over by extremely powerful—and usually elderly—chairmen who have risen slowly through the ranks during their years of residence on Capitol Hill The so-called seniority system requires that committee chairmanships (and the chairmanships of subcommittees) go to the longest-serving—and not necessarily the most able—committee members who belong to the political party which holds the majority of House seats

Each committee has its own area of responsibility—thus, for example, the Armed Services Committee deals with legislation concerning programmes of the Department of Defense and the Agriculture Committee deals with agricultural legislation When a bill is introduced into the House, it is referred to the appropriate committee, which usually assigns it to one of its subcommittees Hearings are held on the bill, it usually gets amended or completely rewritten by the subcommittee before being passed on to the main committee, which can further amend it before it moves on to the full House for further amendment and final passage

The committee therefore represent the centres of power since they shape the legislation which reaches the floor Moreover, committee chairmen (and to some extent subcommittee chairmen) wield a tremendous amount of power over the system since they control the appointment of committee staff, who deal with the nuts and bolts of the committee work and frequently write the legislation themselves And, equally important, the chairmen can kill off legislation with which they disagree simply by keeping it bottled up in their committees, never allowing it to be brought to a vote on the floor of the House

One problem with the system is that it has been more than a quarter of a century since any serious attempt was made to alter committee jurisdictions, and areas of authority have consequently become considerably blurred Take energy research and development, for example The Committee on Science and Astronautics is deep into that subject since it has jurisdiction over the programmes of the National Science Foundation, but so is the Interior Committee, the Public Works Committee, the Commerce Committee and the Joint Committee on Atomic Energy The net result is that there has been considerable jurisdictional turf fighting between committee members for control of energy research legislation

Another effect of confused jurisdictions is that when a member of the Committee on Science and Astronautics, for example, writes a bill aimed at setting up a programme of, say, solar energy research, he shapes the legislation to give the programme to the National Science Foundation or NASA so that his committee gets jurisdiction over it The result is that programmes don't always get assigned to the most suitable agencies

Then there is the problem that Members of Congress often serve on several committees, which considerably dilutes their effectiveness

So, all-in-all, the House of Representatives is not a terribly efficient place, and that's why two years ago a special bipartisan committee, headed by Richard Bolling, a skilled legislator from Missouri, was appointed to recommend some reforms

Almost a year ago, the Bolling Committee came up with a batch of recommendations which immediately threw most of the committee chairmen into fits of apoplexy It suggested a fundamental realignment of committee jurisdictions, that members could only serve on one major committee, and that the powers of the Speaker should be elevated

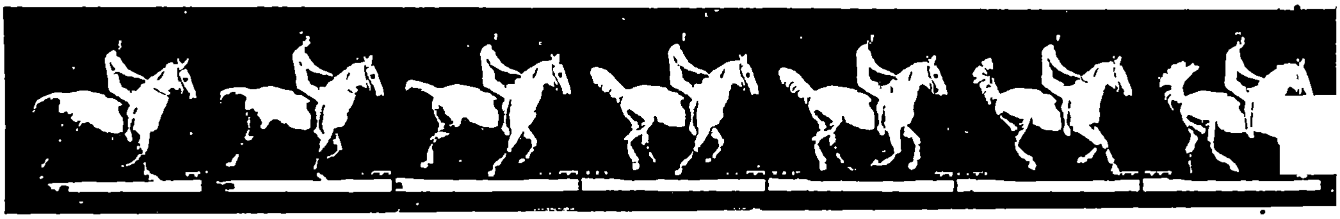
But by far the most controversial provisions it contained were the committee realignments

For a start, the Bolling proposals would have stripped one of the most powerful committees—the Ways and Means Committee—of many of its functions, it would have split the Education and Labour Committee in two, and it would have abolished the Post Office Committee, the Internal Security Committee, and—to all intents and purposes — the Merchant Marine Committee

Not surprisingly, a good number of committee chairmen who had reached the pinnacle of their political careers were not altogether happy about having their power suddenly stripped from them, and they persuaded the Democratic Caucus to appoint another committee to come up with some counter proposals

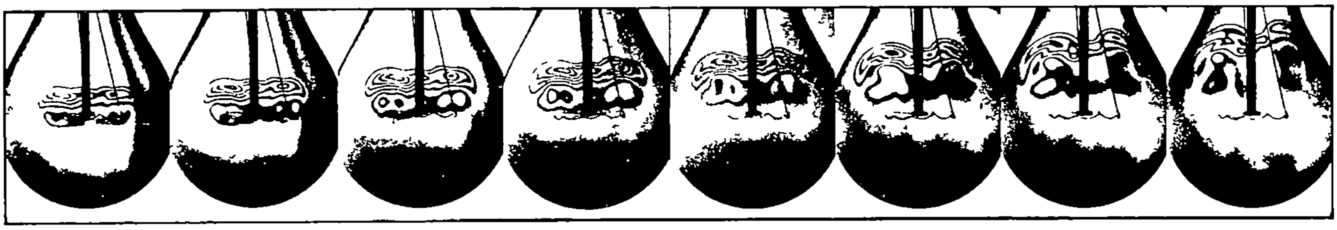
It was those recommendations which were adopted last week, with a few minor modifications, after six days of bitter debate which at times left the House leadership entirely confused

As far as science and technology are concerned, the Bolling Committee had recommended that the Committee on Science and Astronautics should be given authority over all energy research and development, including



Fast workers

EADWEARD MUYBRIDGE (he thought the Anglo Saxon version more striking than plain Edward Muggeridge) was a Victorian photographer who left Kingston-on-Thames in 1852 to sail for America There he was run over by a stage coach and tried for the murder of his wife's lover He was declared insane and acquitted after a former employee had testified "He was most eccentric in his work, he would not take a picture unless the view suited him " On the credit side, he made a stereoscopic record of the Modoc Indian war and toured Central America (as Edwardo Santiago Muybridge), later selling collections of 120 prints at 100 dollars the set, which was a close call to skinning the market at 1876 exchange rates More importantly, Muybridge made photographic studies of movement which laid the foundations of a completely new high speed photographic industry (not to mention a completely new movement in European art) In 1880 he became the first person to photograph movement (using "the famous zoopraxiscope camera") and resynthesise the movement on a screen A strip from this original sequence (above) was featured in an exhibition accompanying the eleventh international congress on high speed photography at Imperial College, London The same congress was marked by an exhibition of applied photography at the Royal Photographic Society, where the very latest achievements of the high speed photographic industry included the series reproduced below It shows the development of a convective flow in the gas filling of an electric bulb as the current through the filament is switched on The photographs are from eight three-dimensional holographic images reconstructed from a sequence of double exposure holograms recorded on a single plate with a pulsed ruby laser The pulse length was about 1 ms and the interval between pulses was 20 ms The pictures were produced by the National Physical Laboratory, with whose permission they are reproduced The Muybridge series appears by courtesy of the John Judkyn Memorial



nuclear energy But the powerful Joint Committee on Atomic Energy objected at such an intrusion on its patch, and the Science and Astronautics Committee eventually wound up with only non-nuclear energy research and development But that is a distinct improvement over the present arrangement in which authority is split over a number of committees, and it also means that the new Science and Technology Committee will get jurisdiction over the non-nuclear programmes of the umbrella Energy Research and Development Administration

The Bolling Committee had also recommended that the science committee be given "special oversight" over military R & D, which would have given it authority to make studies and recommendations on the Pentagon's programmes, but it wouldn't have had the power to write laws in that area But Edward Hebert, the 74-year-old chairman of the Armed Services Committee would have none of that, and his monopoly over the Defense Department programmes remains unbroken

Finally, the Bolling proposals would have given the Science and Astronautics Committee jurisdiction over virtually all the programmes of the National Oceanic and Atmospheric Administration

But Mrs Lenore Sullivan, Chairman of the Committee on Merchant Marine and Fisheries didn't relish the idea of her committee disappearing from under her, and the upshot is a grotesque split with some atmospheric programmes going to the new Science and Technology Committee, while oceanography stays with the Committee on Merchant Marine But at least it was agreed during the floor debate last week that the two committees will work together on oceanic and atmospheric matters

As for environmental research and development, both the Bolling Committee and the Democratic Caucus committee recommended that the new Science and Technology Committee would gain responsibility for that entire area, and so it will

The upshot, then, is that many science and technology matters in the House will now be consolidated into one committee, which should make for smoother operations in some areas But it should be pointed out that the seniority system has survived completely untouched, and nobody had the temerity to alter the workings of perhaps the most powerful committee of all—the House Appropriations Committee, which deliberates on the budgets of the executive departments and agencies

Bolling, at least, remains philosophical about the outcome of his attempts at reform "It is a good start", he said last week, and indicated that he will try again next session

Meanwhile, the Senate has for some time been making noises about taking a look at its own hopelessly confused committee structure, but so far has shown no signs of doing anything about it It is far from clear at this stage, for example, which committees will get jurisdiction over the Energy Research and Development Administration, and science affairs are strung out over a whole range of Senate committees

Since all bills must be approved by both the House and the Senate before they become law, the House reforms are clearly only half-way measures, no matter how good they are ☐

news and views

Fluctuations in climate

THROUGH most of the first part of this century it was widely taken for granted that climate is essentially constant, apart from short-term fluctuations, some of which might involve shadowy cyclic changes In fact, the global climate was at that time changing—a rather general warming and increasing moisture in continental interiors (apart from the Americas)—in ways that made life easier for most people in most places Hence, there was little investigation of the phenomenon Now, however, the decades of neglect have given place to widespread concern over climatic change This is partly because there is some evidence of a global cooling, and a change in the rainfall trends also, setting in from the 1950s onwards There is also a more pressing alarm over the many signs in recent years of an increased range of variability of climate from one year (or short group of years) to another The world population is already so large that there is no margin for even occasional bad years—bad, that is, in the sense of lowered harvest yields in several of the world's principal grain-producing areas Indeed, the world's grain reserves have been reduced each year since 1970 both 1972 and 1974 will rank as bad years in the sense just mentioned, but even in a good year, such as 1971, the reserves fell, and are now under a quarter of what they were before this decade

It was with facts such as these in mind that US Secretary of State Henry Kissinger told the United Nations General Assembly in April 1974

> "The poorest nations, already beset by man-made disasters, have been threatened by a natural one the possibility of climatic changes in the monsoon belt and perhaps throughout the world The implications for global food and population policies are ominous The United States proposes that the International Council of Scientific Unions and the World Meteorological Organisation urgently investigate this problem "

And, in similar vein, Lord Rothschild wrote recently

> " there are several subjects in which I regret that the Think Tank has not so far taken an interest one of these is the effect of the possible changes in our climate on the life of the inhabitants of this island It would, I believe, repay study "

Also, the Climatic Research Unit at the University of East Anglia in Norwich, of which I am director, whose financial plight over the first $2\frac{1}{2}$ years of its existence has several times been mentioned in *Nature*, now has the funds needed to start its work—thanks to the generosity of the Wolfson and Nuffield Foundations in this country and the Rockefeller Foundation in the United States

This issue of *Nature* carries three more in the series of contributions to knowledge on the variations and variability of climate which it has been publishing in recent years Drs Wood and Lovett report on page 594 on the rainfall variations measured in Ethiopia over the last 70 years, and the records of major drought years since AD 1540, analysed in relation to the 11-year sunspot cycles Their result highlights the great range of variation of the annual rainfall in that country within each 10 or 11-year period as likely to have more impact than the longer-term trends, though possibly made more serious by these On page 592 Brown reports a new link between variations in the Earth's magnetic field at the time of sunspot minimum and the strength of the subsequent sunspot maximum, together with the climatic evidence such as that presented by Wood and Lovett this raises the possibility of the use of the Earth's magnetic field to forecast changing weather patterns five or six years ahead

The third contribution, on page 582, is concerned with what can be learnt of the longer record of climatic behaviour from tree rings, in this case the year-rings in spruce (*Picea*) growing in southern Germany Dr Schiegl uses deuterium measurements on the tree rings to show how an indication of a long history of annual mean temperature might be derived This technique is one example of a rapidly increasing number of types and uses of 'proxy' data to extend and corroborate the climatic record for periods before the invention of most meteorological instruments

H H LAMB

Mass extinctions in the fossil record

THE problem of what caused the extinction of particular groups of fossils continues to intrigue both palaeontologist and layman Every schoolboy learns about the dramatic and relatively sudden extinction of the dinosaurs at the end of the Mesozoic Era, and many are the more or less ingenious hypotheses put forward to account for it My own favourite relates the extinction to the relative decline of the gymnosperms or naked seed plants in favour of the flowering plants during the Cretaceous period The surviving conifer and cycad representatives include many producing oils with renowned purgative properties, from which one is drawn ineluctably to the conclusion that the poor dinosaurs died of constipation[1] The trouble with all such hypotheses is their *ad hoc* character, devoted specifically to the dinosaurs Viewed in a broader context, the dinosaurs are seen as but one of a whole series of animal groups, both terrestrial and marine, which died out at the end of the Cretaceous about 65 million years ago An even more spectacular phase of mass extinction, affecting a majority of invertebrate and vertebrate classes both on land and in the sea, took place towards or at the end of the Permian some 160 million years earlier It is no coincidence that the three faunally defined eras of Phanerozoic time, the Palaeozoic, Mesozoic and Cainozoic, are divided by these two so-called crises in the history of life

Attempts to account for these, and lesser, phases of mass extinction can be grouped into two broad categories, involving phenomena either extrinsic or intrinsic to our planet The extraterrestrial explanations have usually centred around the deleterious effect of high levels of cosmic radiation, leading to widespread destruction of organisms, either directly or by damaging genes and thereby preventing successful reproduction Some have argued for episodic pulses of increased radiation compared with that operating at present, for which there is no independent evidence, others have postulated that at times when the geomagnetic field was reversing the 'magnetic protection' of the Earth diminished for period of perhaps as much as a few thousand years, allowing more radiation to penetrate to the surface of the continents and oceans Explanations such as these have fallen out of favour in the last few years for a variety of reasons For example, they have not been able to explain why marine organisms have been affected at the era boundaries more than terrestrial ones, especially plants Furthermore, in the ocean the intensity of radiation diminishes to a small fraction of its atmospheric value only a few metres below the surface

Attention has now turned to intrinsic factors Following the pioneer work of Valentine and Moores (*J Geol*, **80,** 167, 1972) who were the first to seek an explanation for the Permian extinctions by bringing together concepts derived from plate tectonics and modern ecological theory, attempts adopting a similar approach have been made recently to account for the 'crises' at the close of the Mesozoic and Palaeozoic

Hays and Pitman (*Nature,* **246,** 18, 1973) have speculated along the following lines In the late Cretaceous there was a huge marine transgression over as much as a third of the continents, when sea level was raised hundreds of metres as a result of uplift of the mid-oceanic ridges This was a consequence of an acceleration in the rate of seafloor spreading for which there is apparently good independent evidence The transgression was instrumental in inducing a more stable, equable world climate, which led to significant diversification of many animal groups including the dinosaurs and, in the marine realm, reef corals, rudistid bivalves and planktonic foraminifera Highly diverse, stenotopic organisms in stable environments are vulnerable, however, to even slight environmental change The pronounced marine regression at the end of the Cretaceous, related by Hays and Pitman to deceleration of seafloor spreading rates, induced an episode of increased thermal gradients, seasonal temperature contrasts and storminess, together with a significant change in oceanic circulation patterns This sudden decrease in environmental stability was sufficient to cause widespread extinction, affecting the groups cited above and many others The dinosaurs' unsatisfied need was not so much for laxatives as for winter woollies[1]

A significant relationship between the late Permian extinctions and fall of sea level is also inferred by Schopf (*J Geol*, **82,** 129, 1974) His survey disclosed that the number or marine invertebrate families halved from the beginning to the end of the period while at the same time the area of shallow epicontinental seas diminished by rather more than half The lowering of sea level was probably a consequence of the creation of the supercontinent Pangaea as separate continents collided When this happened the seafloor spreading rate diminished as a 'brake' was inevitably applied to plate movement There is no satisfactory explanation of why sea level began to rise again in the early Triassic, although Pangaea did not begin to disintegrate, with the creation of a new spreading axis between Africa and North America, until the early Jurassic In the earlier hypothesis of Valentine and Moores, the extensive late Permian extinctions were attributed partly to increased interfaunal competition as formerly isolated continental shelves were joined together, and partly to a reduction in environmental stability consequent upon the creation of one large continent from several smaller ones (thus, seasonal climatic contrasts would have increased) Schopf draws attention to another factor

Ecologists recognise an exponential relationship between species number and area of the habitat occupied Modern theory has it that animal populations in a given region are in a condition of dynamic equilibrium, with rate of immigration being balanced by rate of extinction The theory has been applied with great success to oceanic islands, which form an excellent laboratory for ecologists Small islands cannot be held to possess 'impoverished' faunas, as formerly thought, they can only support small populations and consequently extinction rates are higher It is a big jump from species abundance in tiny islands to familial abundance in seas bordering whole continents, with rate of immigration being replaced by rate of origination through evolution, but Schopf is prepared to take this bold step He can at least claim the support of a leading researcher in the new ecology (D Simberloff, *J Geol*, **82,** 267, 1974), who has erected a model for the Permian diversity reductions and extinctions based on reasonable biological assumptions, which shows a good accord with Schopf's data Thus merely reducing the area of the Permian shelf seas might be sufficient in itself to explain the increased rate of extinction, reaching a maximum at the end of the period

An attractive feature of the two hypotheses outlined above is that they account for changes in organisms inhabiting a wide variety of habitats in a way which is consistent with modern ecological theory, and provide a satisfying correlation with independently deduced geological events of great significance In both cases the fundamental control on the extinctions is likely to have been variation in heat flow from the mantle, affecting the density, volume and spreading rates of oceanic ridge systems Though many important questions remain unanswered, an explanation along these lines is much less of a *Deus ex machina* than that involving variations in cosmic radiation

A HALLAM

Neural hypothesis of muscular dystrophy is flourishing

MANY workers in the field of neuromuscular diseases will feel that there is more evidence in favour of the neural hypothesis of muscular dystrophy than Professor Bradley's article[1] would imply Although it is true that Harris and Marshall[2] did not find evidence of functional denervation in dystrophic mouse muscle, the earlier and contrary observations of McComas and Mrozek[3] have now been confirmed by Law and Atwood[4] Fibrillation potentials, usually regarded as a sign of denervation, are also a feature of murine dystrophy[5] Possible ultrastructural correlates of synaptic dysfunction have been found in the axon terminals of dystrophic mice in studies from two laboratories[6,7] Even if Harris and Marshall were correct, these authors demonstrated nonetheless that the neuromuscular junctions of dystrophic mice were unusually susceptible to adverse conditions within the experimental milieu

Dr Salafsky[8] was one of the first to transplant minced muscle between normal and dystrophic mice and, by controlled experiments encouraging reinnervation of the transplants, was able to study their contractile properties The explanation offered by Dr Bradley is insufficient to account for all his findings Of those muscle transplantation studies in which care was taken to permit reinnervation, two[9,10] have largely confirmed Salafsky's work and one[11] has not

In the cases of human Duchenne dystrophy the evidence is mixed Fibrillation potentials certainly occur[12] and the work of Desmedt and Borenstein[13] is cited as indicative that denervated muscle fibres become reinnervated In contrast, a careful electron-microscopical study by Dr Andrew Engel and his associates[14] has not yielded any evidence of structural abnormality in the motor axon terminals This result, and the finding of normal numbers of motoneurones at autopsy[15], does not contradict the neural hypothesis as originally proposed[16] Indeed, recent studies with A Upton and P Jorgensen, as yet unpublished, strongly suggest that it is possible for motoneurones to have 'silent' synapses In patients with unequivocally neuropathic disorders it now seems that neuromuscular junctions may become inexcitable but still capable of transmitting a neurotrophic influence to the muscle fibres If this interpretation is correct, it is not difficult to conceive of more severe dysfunction in which the synapses, although present, can no longer subserve a trophic action

So far as the motor unit counting results are concerned, the necessary electrophysiological technique was first applied by McComas, Sica and Currie[17] to the extensor digitorum brevis (EDB) muscle The choice of muscle was subsequently criticised on the grounds that the motor nerve was vulnerable to trauma, particularly in disabled patients such as those with dystrophy[18,19] This criticism would seem unfounded if the recent reports[20,21] of normal numbers of motor units in Duchenne dystrophy are correct Our own solution to the problem of trauma was to study patients with very early stages of the disease and to extend the technique to muscles other than EDB These last results[22] are in keeping with the earlier ones Since the full papers by Panayiotopoulos *et al*[23] and by Ballantyne and Hansen[24] have yet to appear, it is difficult to be certain that these workers have achieved the refinements of the counting technique reported by Bradley Scarpalezos and Panayiotopoulos[20] in their brief description claim to be able to detect very small muscle responses "overlapping at noise level" on single, rather than superimposed, oscilloscope sweeps Not only is this feat a logical impossibility, but the value of the superimposition technique (employed by ourselves) in detecting very small evoked biological signals has been accepted ever since the pioneering experiments of Dawson[25] As practised by our own group, the motor unit counting technique has proved itself as a sensitive and reliable means of detecting neuropathic disorders and it is gratifying that Ballantyne and Hansen[24] have obtained almost identical values for healthy subjects It is important to add that the technique has recently been employed in a 'blind' study of 17 possible cases of malignant hyperthermia (unpublished work with B A Britt and W Kalow) and has so far been completely accurate in predicting the status of the subjects

I feel that the most direct evidence against a neural aetiology of dystophy is the parabiotic cross-innervation experiment of Douglas and Cosmos[26] in mice The strongest evidence against a myopathic aetiology (though not necessarily for a neural one) is the exceedingly elegant chimaera study of Peterson[27] At first sight contradictory, these observations may be reconciled by taking into account the stage of development of the animal at which the foreign nerve was introduced to the muscle Thus, it is possible that the very first contact between a genotypically dystrophic motoneurone and a genotypically normal muscle fibre in the embryo will commit the latter to a dystrophic growth thereafter, if so, any later experimental manipulations with innervation would be ineffective

Regardless of the true state of play, the neural hypothesis has successfully drawn attention to the possibility of deranged trophic mechanisms being present in neuromuscular disease Whether or not the hypothesis is eventually shown to be correct, it will have served a useful function if it advances our understanding of dystrophy or brings a cure one step closer

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Drilling in the Antarctic

from Peter J Smith

So much attention is given to the large-scale geological programmes, such as the Deep Sea Drilling Project, that there is a tendency to overlook the more modest investigations which face technical and logistic problems scarcely less formidable A good case in point is the three-nation programme of drilling in the McMurdo Sound region of Antarctica By the late 1960s it was clear that if significant progress were to be made in investigating the geology, geochemistry, glaciology, lake-bottom stratigraphy and thermal characteristics of the dry valleys west of McMurdo Sound, it would be necessary to supplement surface studies with borehole data Accordingly, several drilling proposals were submitted to the National Science Foundation which coordinated all acceptable proposals into a single programme with participating scientists from the United States, Japan and New Zealand Thus was born the Dry Valley Drilling Project (DVDP), since expanded to include Ross Island and McMurdo Sound itself

The original aims of the DVDP as described by McGinnis *et al* (*Antarctic J* , **7,** 53, 1972) included studies of palaeontological and volcanic evolution during the poleward migration of Antarctica palaeomagnetic reversals, the geochemistry of polar desert soils and permafrost, heat flow, the hydrogeology of ice cap margins and the global tectonic significance of the McMurdo volcanics although it is already clear that the first of these objectives will not be met An initial exploratory season was envisaged, followed by three drilling seasons involving a minimum of 10 holes During the exploration in the 1971-1972 summer season electrical depth soundings were taken to determine permafrost thickness and the nature of the material beneath the permafrost, seismic refraction profiles were run in lake basins to establish the relief and structure of bedrock and overburden thicknesses, and a regional aeromagnetic survey was made to provide structural control. In addition, environmental monitoring (physical and microbiological) was begun and will be continued throughout the project.

Drilling began during the 1972-1973 season with two holes through the volcanic rocks of Ross Island; and preliminary geological and geochemical results from these sites (boreholes 1 and 2) were described about a year ago (*Antarctic J.*, **8,** 157; 1973). During the 1973-1974 summer, seven more holes were drilled in the Ross Island volcanics (borehole 3 adjacent to hole 2) and in the dry valleys at Lake Vanda (boreholes 4 to 9); and a series of reports on this work has just been published (*Antarctic J.*, **9,** 125; 1974).

According to Kyle and Treves the cores from holes 1, 2 and 3 reveal that the geological history of Hut Point Peninsula (Ross Island) is much more complex than the surface geology suggests, which is itself sufficient vindication of drilling. The oldest unit penetrated by holes 2 and 3 is a 200+ m thick pile of hyaloclastite representing early eruptive events that took place below ice or water; in other words, an early stage of marine volcanism involved the construction of a hyaloclastite pedestal which may have impinged on a thick ice shelf covering the Ross Sea more than 1.2 million years ago. The higher lavas, on the other hand, are apparently subaerial flows and pyroclastic units which represent a single differentiation series starting with olivine–augite basalts, working through augite–kaersutite basalts and ending with kaersutite hawaiites, although the phonolites on an adjacent hill may well be more extreme differentiates of the same magma chamber.

The cores from the dry valleys not unexpectedly comprise glacial and marine sediments, although two of the holes also penetrated the crystalline basement. Both sedimentary and igneous cores are still under laboratory investigation so few geochemical and mineralogical results are available. But Tarii reports that stable isotope studies have already revealed the sources of core ice; in Lake Vanda, for example, most of the present water apparently originates as fresh water whereas deeper sedimentary layers are still under the influence of sea water. Also Gumbley *et al.* have begun to use the upper few metres of sediment from Lake Vanda to trace the lake's Late Quarternary history.

The DVDP can already be credited with the resolution of at least one long-standing disagreement. Over a decade ago, Armitage and House (*Limnol. Oceanog.*, **7,** 36; 1962) discovered that although Lake Vanda lies in a region where the mean air temperature is −18° C, it has a bottom water temperature of +25° C. This led Armitage and House, and later Angino *et al.* (*Sci. Bull.*, **45,** 1097; 1964), to suggest that below the lake there are either high geothermal gradients or hot springs.

Wilson and Wellman (*Nature*, **196,** 1171; 1962) ruled out hot springs on the grounds that the measured isotherms in the lake are nearly horizontal. Not only are hot springs unlikely in Antarctica because the great thickness of frozen ground precludes abundant groundwater; they argued that the entrance of springwater into the lake in conjunction with any possible hot spring would produce a much more complicated thermal pattern. Instead, they developed a theory of solar heating in which solar energy penetrates the lake's ice cover (found to be extremely transparent) and is absorbed in the water below. In support of this view, Wilson and Wellman pointed to the extreme clarity of the water and to the decrease in temperature gradient with depth (which implies a heat 'source' in the water itself). Heat flow measurements in the upper 30 cm of lake sediment also seemed to show that heat is flowing from the water to the sediment. But Ragotzkie and Likens (*Limnol. Oceanog.*, **9,** 412, 1964) produced precisely the opposite result from similar measurements and therefore attributed the high bottom temperature to a combination of solar heating and high geothermal gradient.

Wilson *et al.* have now resolved this question by making thermal measurements in DVDP hole 4 which penetrated the crystalline basement below Lake Vanda. The temperature in the basement 15.5 m below the lake bottom is consistently 0.48° C lower than that in the sediment 0.5 m below the lake bottom. The corresponding temperature gradient (average 0.032° C m^{-1}), combined with estimates of thermal conductivity, shows that Lake Vanda is losing heat downwards at a rate of 0.5–1.0 cal cm^{-2}s^{-1}, thus convincingly supporting the view that geothermal heat is not the reason for the lake's high temperature.

Eltanin bailed out

To oceanographers the name Eltanin probably stands second only to Glomar Challenger. From 1962 to 1972 this vessel carried out geological, geophysical, geochemical, biological and meteorological researches which covered some 80 per cent of the southern ocean between 35° S and Antarctica. Then a $1.5 million budget cut in the US Antarctic Research Program ended its active work.

But now the Eltanin is about to begin a new five-year programme on Antarctic research as a result of an agreement between the United States and Argentina. The ship, renamed Islas Orcadas and operated from Buenos Aires by the Argentine navy, will carry out joint scientific expeditions with support from the National Science Foundation and the Argentine National Antarctic Directorate.

Corvine cannibalism

from our Animal Ecology Correspondent

ARGUMENTS have been raised for years about the functions and consequences of territories to animals. Since the ultimate restraint to population increase is availability of food, one might expect the relationship of territory size to food to be both positive and linear. For some species, mostly herbivores, this may be true or nearly so. But often for both carnivores and herbivores territory size is unrelated to food supply (Watson and Moss, in *Animal Populations in Relation to their Food Resources*, 167, Blackwell, Oxford, 1970). Hinde points out that territories have complex functions with consequences both harmful and advantageous to an individual's chance of breeding success (*Ibis*, **98,** 340; 1956). Simple answers cannot be expected to complex questions.

In a well designed series of field experiments on carrion crows in north-east Scotland, Yom-Tov added extra food to the environment to ascertain if there was a direct relationship between territory size, food supply and breeding success (*J. Anim.Ecol.*, **43,** 479; 1974). There was circumstantial evidence that there was no absolute food shortage during the breeding season. Food in the form of hens' eggs and dead hens' chicks was placed near to artificial trees both within and without established crow territories. This treatment failed to increase the breeding density of adults. But one egg and five chicks offered daily to a group of fourteen breeding pairs in the close proximity of their nests resulted in a significantly higher survival rate of nestlings although there was no difference in clutch size. At fledging an average of 2.3 young had survived in the experimental nests compared with 1.1 in the controls.

The other effect of food added daily from the start of the year was to significantly shift the date of the start of laying, bringing it forward by 5 days. Earlier layed clutches were almost twice the size of late clutches, but the number of fledglings produced per nest was about half. So increased winter food affected date of laying but not, *per se,* the number of fledglings produced per nest.

How does extra food increase the survival rates of nestling crows? Predation of the eggs and desertion by the adults were the main factors responsible. Krebs has pointed out that artifically increased winter food has no effect on the number of territorial breeding pairs of great tits during the following spring (*Ecology,* **52,** 2; 1971). Those nests that were closer together than 45 m suffered 12% more predation (mostly by weasels) than did those nests spaced over 45 m apart. Carrion crows are their own chief predators—they prey to a significant extent upon the eggs and nestlings of their own kind. With cannibalism as the regulatory mechanism, the ultimate limiting factor for the number of nestlings reared is the dispersion of food within the territory. An abundance of food close to the nest means that the clutch is left unattended for shorter intervals than if the food is widely scattered. To remain near the nest breeding crows limit their territory size. The lower limit to territory size depends upon the amount of food available and the upper limit depends upon its dispersion and the ability of the pair to defend its nest.

Cyclic AMP and pattern formation

from Paul Epstein

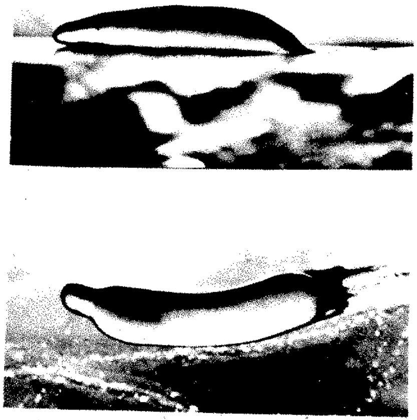
By staining with fluorescent antibody specific for cyclic AMP, Pan *et al.* (*Proc. natn. Acad. Sci. U.S.A.,* **71,** 1623; 1974) have been able to observe the distribution of cyclic AMP in both the unicellular amoebae and the multicellular pseudoplasmodium of *Dictyostelium discoideum* and several related species of cellular slime mould. Cyclic AMP was first shown to be important in the life cycle of *D. discoideum* when it was isolated as the naturally occurring acrasin, or chemotactic agent by which the vegetative amoebae signal each other to aggregate (Barkley, *Science,* **165,** 1133; 1969). Now the studies by Pan *et al.* suggest that cyclic AMP is important not only for aggregation, but for determination, or pattern formation, as well.

In the cellular slime moulds, once the aggregation process is completed, the amoebae form a pseudoplasmodium, or grex, which, depending on environmental conditions, may migrate for an indefinite period before beginning to differentiate into two morphologically and functionally distinct cell types—spore cells and stalk cells. By staining with vital dyes, Bonner (*The Cellular Slime Molds, second ed.,* Princeton University Press, Princeton, New Jersey, 1967) showed that the cells in the anterior third of the grex become stalk cells, whereas the cells in the remaining posterior portion become spore cells.

The cells in the grex are not predetermined, however, since a grex which is sliced transversely can form two normal fruiting bodies, containing the normal proportions of spore and stalk cells (Raper, *J. Elisha Mitchell scient. Soc.,* **56,** 241; 1940). This observation indicates that the cells can recognise their position within the grex, and somehow alter their developmental fate accordingly.

How do the cells know their position in a field? One theory is that they respond to gradient(s) of concentration of small molecule(s) (Wolpert, *J. theoret. Biol.,* **25,** 1; 1969). More recently, McMahon developed a theory of pattern formation using *D. discoideum* as a model, which holds that a sharp concentration boundary of a small molecule can develop through its regulation by contact-sensing molecules on the plasma membranes of cells in the field (McMahon, *Proc. natn. Acad. Sci. U.S.A.,* **70,** 2396; 1973). These regulatory molecules would be the macromolecules which synthesise and degrade the small molecule. In this case, the polarity of the morphogenetic field is determined by the boundary of the small molecule, which in turn is determined by the distribution of contact-sensing molecules.

Although one theory invokes a gradient and the other a boundary, both models require non-uniform distribution of a small molecule to determine the pattern of development. The results of Pan *et al.* suggest that cyclic AMP might be one such small molecule which determines pattern formation in *D. discoideum.* Pan *et al.* showed that as the pseudoplasmodium was allowed to migrate, a distribution of cyclic AMP arose such that the higher concentration was found in the anterior, pre-stalk region. In some cases, they observed an abrupt boundary of cyclic AMP concentration between the anterior and posterior portions of the grex, as theorised by McMahon.



Migrating grex of *Dictyostelium discoideum* seen from the side and from above. From *Developmental Biology* by Nelson Spratt.

It is interesting to consider two earlier observations which bear on pattern formation and differentiation in *D. discoideum,* and to see how they fit with the idea that pattern formation in the grex is determined by the distribution of cyclic AMP. Bonner (*Proc. natn. Acad. Sci. U.S.A.,* **65,** 110; 1970) subjected isolated amoebae to high concentrations of cyclic AMP and found that they developed into stalk cells. From this observation, he concluded that cyclic AMP functions as an inducer of stalk cell differentiation. The observation of Pan *et al.* is consistent with this conclusion.

The other observation was made by Raper (*J. Elisha Mitchell scient. Soc.,* **241,** 1940), who sliced a pseudoplasmodium transversely into four sections. Section one, the anterior tip, could be made to migrate for various times. If induced to begin fruiting body formation immediately, without any resumption of migration, this section developed into an extremely abnormal fruiting body, bearing very few or no spores, and an excessively heavy stalk. If 3–6 h of migration were allowed before fruiting formation began, the resulting structure was less abnormal but still bore fewer spores and more stalk. If 24 h of migration were allowed, the tip gave rise to a completely normal fruiting body, with the correct proportion of spore and stalk cells. Hence, Raper demonstrated that the cells at the anterior tip of a pseudoplasmodium are determined to become stalk cells, and that this determined state is altered progressively as the tip migrates.

In accord with this observation, Pan *et al.* found that the distribution of cyclic AMP in the grex becomes more apparent as it migrates. Likewise, McMahon, in his theoretical treatment, showed the boundary of cyclic AMP concentration arising near one end of the field, and moving along the field with time. McMahon calculated that by about 3–5 h the boundary becomes stabilised at a position one-third of the way down the field; that is, at the position which would give rise to the normal proportion of spore and stalk cells.

Although substantial proof is required before one can state that cyclic AMP is responsible for pattern formation in *D. discoideum,* the results of Pan *et al.* at least suggest such a function. If further studies provide proof, this would represent the first identification of a small molecule responsible for pattern formation. ●

Clustered genes and non-transcribed spacers

from Benjamin Lewin

MOST eukaryotic genes are probably present in only one copy for each haploid genome Two notable exceptions to this rule are provided by the genes coding for histone proteins and those which specify ribosomal RNAs here many genes, apparently identical for ribosomal RNA genes and with little variation in the histone genes, are organised in a cluster in which each transcribed sequence seems to be separated from the next by a non-transcribed spacer region The structure and function of the spacer are presumably relevant to the control of gene activity, and perhaps to the problem of the suppression of variation in the multiple copies of each transcribed sequence

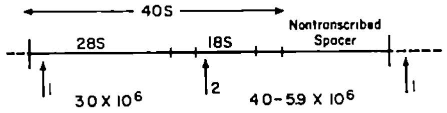
During oogenesis in *Xenopus laevis* the nucleoli which contain ribosomal DNA are amplified to become a major cell component The ribosomal DNA can thus be isolated in bulk and has been the subject of much work on sequence organisation This DNA was recently used as a substrate for the *Eco*RI endonuclease of *Escherichia coli* by Morrow *et al* (*Proc natn Acad Sci U S A* **71**, 1743–1747, 1974) who found that each repeating unit (transcribed plus non-transcribed) suffers two cuts When *X laevis* rDNA molecules of about 50×10^6 daltons were treated with excess *Eco*RI enzyme the principal fragments had weights of $3 0\times10^6$ and $4 2\times10^6$ daltons, prominent among the minor fragments were two of $3 9\times10^6$ and $4 8\times10^6$ daltons These products were annealed with DNA of the plasmid pSC101 (which is cleaved at a single site, generating a linear molecule from its circular genome) to give plasmid–rDNA recombinant molecules that were then replicated by growth in *E coli*

When the recombinant plasmid DNAs were isolated from several bacterial cell lines and analysed by cleavage with the *Eco*RI restriction enzyme, each recombinant plasmid gave fragments with sizes typical of the linear molecule of pSC101 and of the cleavage products of *Xenopus* rDNA

By hybridisation with 18S and 28S *Xenopus* rRNA, Morrow *et al* showed that the hybrid plasmid CD4, which contains both the $3 0\times10^6$ and $4 2\times10^6$ cleavage fragments, anneals equally well with both rRNAs, the CD18 plasmid containing only the $3 0\times10^6$ dalton fragment anneals principally with 28S rRNA while the DNAs of plasmids CD30 and CD42, which contain the $3 9\times10^6$ and $4 2\times10^6$ dalton fragments respectively, react mostly with 18S rRNA These results suggest that the tandem repeats in the rDNA are heterogeneous, each consisting of the $3 0\times10^6$ dalton sequence (which contains the 28S rRNA sequence) and the $3 9\times10^6$ or the $4 2\times10^6$ dalton sequence (containing the 18S rRNA sequence and presumably differing by the presence or absence of an extra sequence, other variations in this region are suggested by the existence of other size fragments)

Since the transcribed region of *Xenopus* rDNA takes only one form, heterogeneity in sequence must be ascribed to the non-transcribed spacer region, a conclusion supported by the report of Wellauer *et al* in the *Proceedings of the US National Academy of Sciences* (**71**, 2823–2827, 1974) By cleaving *Xenopus* rDNA with the *Eco*RI enzyme, they were able to isolate two classes of fragment on agarose gel electrophoresis Every preparation contains a prominent band of $3 0\times10^6$ daltons and several bands are present in lesser amounts which vary from 4 0 to $5 8\times10^6$ daltons To decide whether the heterogeneity of the larger bands exists within single genomes or reflects differences between animals, they examined the rDNA from a single nucleolus organiser, this contained at least three of the larger size classes as well as the $3 0\times10^6$ dalton band The repeat length of transcribed plus non-transcribed *Xenopus* rDNA is about 8×10^6 daltons, clearly implying that the repeats must be heterogenous in length (since otherwise the total length of fragments would equal rather than exceed the repeat length) When the duplex lengths of the fragments were measured by electron microscopy, the smallest proved to have a homogenous size of $3 0\times10^6$ daltons and the number of these small fragments equalled the sum of the number of the larger fragments Each repeat therefore seems to comprise one 3×10^6 dalton region and one of the larger regions

Secondary structure mapping has previously been used by Wellauer and David (*Proc natn Acad Sci U S A* **70**, 2827–2831, 1973) to order the sequences for ribosomal RNA in the precursor of the mature rRNA (see Lewin, *Nature*, **250**, 619–621, 1974) When RNA is spread for electron microscopy, hairpin loops may form between short complementary sequences and the position of each loop along the linear molecule provides a map of it The 28S rRNA has a characteristic map present at the 5′ end of the precursor and the 18S rRNA is characterised by an absence of loops Wellauer *et al* now extend this technique to DNA which has been denatured to single strands The small $3 0\times10^6$ dalton fragment cleaved from *Xenopus* rDNA by the *Eco*RI enzyme is homogeneous in structure, it seems to result from cuts made by the enzyme about $0 3\times10^6$ daltons into the 28S rRNA sequence and about $0 2\times10^6$ daltons into the 18S rRNA sequence, this fragment thus represents the starting region of the rRNA precursor, lacking the immediate 5′ end The other fragment generated by these two cuts has one end devoid of structure, presumably corresponding to the 18S rRNA sequence, with the other end displaying the structure typical of the very beginning of the 28S rRNA structure



Summary by Wellauer *et al* of structure of an rDNA repeat unit

The central regions of all larger fragments show a related structure, but vary in length It is therefore the non-transcribed spacer region in which the heterogeneity resides and the formation of heteroduplexes suggests that the shorter large fragments represent deletions of sequences present in the longer large fragments How this heterogeneity relates to the functions of the nucleolar rDNA is not yet known, one reservation about the interpretation of these results, however, is that the source of the rDNA is the amplified material of the oocyte and it remains to be proven whether the same situation is found in the somatic cell

Analysis of histone genes is at a less advanced stage than that of ribosomal RNA genes Each of the five histone proteins seems to constitute a unique amino acid sequence in any species, and the hybridisation analysis of Weinberg *et al* (*Nature*, **240**, 225–228, 1972) suggested that their messenger RNAs are coded by genes repeated some 500–1,000 times in the genome of the sea urchin *Psammechinus miliaris* Because of the multiple codons representing each amino acid, identity of protein sequences need not imply identity of the repeated nucleic acid sequences coding for them, but the hybridisation analysis suggests only limited heterogeneity The histone messengers of another sea urchin, *Lytechinus pictus*, have been separated by gel electrophoresis into a small number of groups by Grunstein *et al* (*Cold Spring Harb Symp quant Biol*, **38**, 717–724 1973) and a fingerprint analysis of one of these messengers is consistent with the idea that its sequences are fairly uniform

The histone genes of *Psammechinus miliaris* band on CsCl gradients at a density only slightly greater than that of bulk DNA, but Birnstiel *et al* (*Proc* *natn Acad Sci U S A*, **71**, 2900–2904, 1974) now report that they can be isolated by their ability to bind preferentially to actinomycin C_1 which causes them to form a low density band The band isolated in this way hybridises well with a preparation that contains the messengers for four histones (mRNA activity for histone F_1 has not yet been demonstrated), since each of the four histone messengers shows a virtually identical reaction with the fractions of DNA separated on the CsCl–actinomycin gradient, it is likely that the different histone genes are intermingled rather than clustered in four individual groups

Comparison of G+C content of histone DNA and RNA, and the results of shearing and of melting histone DNA all suggest that the histone gene cluster also contains other sequences that are poorer in G+C content The release of the histone-coding from the other sequences only by shearing suggests that both components are integral parts of the gene cluster The melting curve of renatured DNA from the preparation implies the presence of some heterogeneity in sequence, probably in the sequences which do not code for histones Whether the component poor in G+C represents a non-transcribed spacer region analogous to that of the rDNA gene cluster is not of course revealed by present data but this is one of the functions that can be imagined for it

Two by two

from D H Jennings

It is rare these days to attend a conference covering the whole spectrum of biology But the Society for Experimental Biology mounted such a conference—on symbiosis—at Bristol on September 2–6 By retaining the original de Bary definition, namely that symbiosis is the association of two different organisms, it was possible to have all shades of interest from molecular to whole organism biology represented Thus, ecologists, though not contributing directly, could find much to interest them Indeed, two contributions were particularly seminal for ecological studies Fricke (Max-Planck-Institut, Seeiweesen) indicated how behavioural studies of organisms in the Red Sea can provide an understanding of animal interactions in nature thus adding flesh to the bare bones of numerical population studies Cox (Kings College, London) discussing his work on intraerythrocytic parasites, pointed out that population biologists cannot continue to think about particular species without considering their parasites He showed how susceptibility to various virus diseases can be dramatically modified by the presence of parasites

There was much emphasis on how the invading symbiont avoids attack by its potential host Terry (Brunel University) and Smithers (National Institute for Medical Research, Mill Hill) presented their elegant studies on the evasion of the immune response by *Schistosoma* which can survive by its ability to acquire host molecules Muscatine (University of California, Los Angeles) provided data indicating the recognition by *Hydra* of the appropriate *Chlorella* cells takes place after engulfment at the stage when the algal cells move to the base of the digestive cells Studies on symbiosis in *Paramecium* are now also beginning to yield relevant information with respect to bacteria (Preer, University of Indiana, Bloomington) and *Chlorella* (Karakashian, Max-Planck-Institut, Wilhelmshaven)

In spite of the decision to use the de Bary definition, there was much discussion of what is meant by the term 'symbiosis' Mortimer Starr (University of California, Davis) produced a new classificatory scheme which has the virtue of both clarifying thinking about the phenomenon and indicating where further experimental work is required—to decide, for example, whether a symbiotic association is mutualistic or obligately parasitic In this respect, Smith (University of Bristol) put those interested in lichens further in his debt by his careful analysis of the present data in the field which indicate that there is no detectable benefit to the algal partner Also Coffey (Trinity College, Dublin) presented further information about the rust fungi, once thought to be the classic example of obligate pathogens in plants but which can now be grown in culture

Many problems are emerging for the biochemists, particularly on how a symbiont affects the physiology of the host which it has invaded Biochemists should be impressed by *Aplysia* which does a far better job than they of isolating chloroplasts There is no doubt, as shown by Trench (Yale University), that these chloroplasts are fully functional, though the relationship between these organelles and the animal cytoplasm in which they reside is not the same as that which exists in the intact cell from which the chloroplasts are extracted

But biochemists and molecular biologists have other reasons for being interested in symbiosis Chloroplast symbiosis is, in a number of ways, the living expression of how eukaryotic organelles are believed to have arisen, namely by successful invasion of a primitive glycolytic prokaryote by oxygen utilising (to give mitochondria) and photosynthesising (to give chloroplasts) prokaryotes Though the theory has a respectable age, Lynn Margulis (University of Boston) has done the most recently to bring it up to date In a debate on the theory she propounded her views with infectious enthusiasm But though most of the audience were believers, Raff (University of Indiana, Bloomington) in a careful comparison of bacteria and mitochondria showed that their faith may be blind

Five hundred million years ago in Wales

from I Strachan

About half of those attending the symposium organised by the Palaeontological Association of London on the Ordovician System (September 17-20) had spent the previous week touring Wales looking at the classic geological sites of Arenig, Llandeilo, Caradoc and Bala

Much of the material presented at the meeting in Birmingham was factual and included new information as well as regional summaries, but there was also a considerable questioning of current dogmas F J Fitch (Birkbeck College, London) and others speaking on Ordovician geochronology pointed out the possible sources of error inherent in radio-isotopic dating, particularly in Lower Palaeozoic rocks, and stressed the need for the use of standard reference scales (such as that produced by the Geological Society of London in 1964) so that data from different sources could be accurately compared They concluded that a length of 65 Myr (between 510 and 445 Myr ago) was reasonable for the Ordovician, compared with 70 Myr for the preceding Cambrian and 40 Myr for the succeeding Silurian

Given this broad time span, it was not surprising that the details of the regional pictures presented by later speakers should show some differences The biostratigraphical problems were analysed in various groups of fossils W B N Berry (University of California) related his graptolite faunas to warm and cool water masses using a palaeogeographical reconstruction with the land all on one hemisphere M Lindstrom (University of Marburg) felt that the conodont faunas agreed in provincialism with the graptolites but C R C Paul (University of Liverpool) found distinct North American, Baltic and Mediterranean cystid faunas only up to the lower part of the Caradocian, after which time provincial boundaries became fuzzy This he related to closing up of ocean basins during late Ordovician plate movements.

He also discussed the functional aspects of respiration in cystids to critically low oxygen levels in shallow tropical seas. This linked the palaeogeographic approach to the climatic one which was discussed by N. Spjeldnaes (University of Aarhus, Denmark). The now well known glacial deposits of the Moroccan Anti-Atlas were beautifully illustrated by J. Destombes (Service géologique, Rabat) and other speakers referred to climatic cycles in the Ordovician which influenced the occurrence of various groups of fossils. Changes in faunas could be caused as much by climatic changes as by migration between continental plate margins and the disentangling of plate movements through the whole length of the Ordovician needs much further work.

None of the formal papers included any discussion of the boundaries of the Ordovician, most speakers making it clear whether they regarded the Tremadocian as Cambrian (as in English usage) or Ordovician. But the large gathering of experts from so many countries, including the Soviet Union, provided the opportunity for meetings of the Commission on Stratigraphy of the International Union of Geological Sciences. Several recently formed subcommissions and working groups of the commission will have a great deal to do with correlation of strata between countries and continents. Differences of interpretation were much in evidence during the closing general discussion in Birmingham, particularly between those based on groups of fossils traditionally used, such as brachiopods and trilobites, and those which have come into prominence in the last few years, such as conodonts. Some of the ideas were certainly provocative and augur well for the future liveliness of the subject. As an eminent worker on Palaeozoic gastropods put it to close the meeting—a snail never gets anywhere unless he sticks his neck out.

Expanding wings in moths

from our Insect Physiology Correspondent

EVERY lepidopterist knows that emerging butterflies and moths go through a characteristic sequence of wing-folding movements, with wing expansion and general hardening of the cuticle, before settling down into their normal resting posture, and that the force for wing expansion is the hydrostatic pressure of the blood generated by strong contraction of the abdomen. Truman and Endo (*J. exp. Biol.*, **61**, 47, 1974) have now elucidated the complex of physiological factors engaged in this familiar phenomenon.

It was shown by Fraenkel many years ago that if the newly moulted blowfly is obliged to continue burrowing through the soil, it remains soft with the wings and body unexpanded for many hours. Only when it makes its escape is the cuticle expanded, hardened and darkened. It was generally assumed that this hardening process must be controlled by a hormone, but that was demonstrated by Cottrell only in quite recent years and confirmed by Fraenkel and Hsiao, who named the hormone in question 'bursicon'. It is liberated from the brain and other ganglia of the nervous system.

Truman and Endo found that the emerging adult of the tobacco hawkmoth *Manduca* behaves in the same way. It likewise pupates in the soil, and so long as the emergent moth is confined it shows intense digging behaviour, and the spreading of the wings is suppressed, sometimes for 24 hours. The stimulus which maintains the inhibition comes from the head: decapitated moths spread their wings immediately, regardless of whether they are confined. Moths decapitated at the very moment of eclosion, however, show neither wing inflation nor wing folding. But if decapitation is delayed until five seconds after emergence, the full wing-spreading behaviour occurs.

This effect of the brain is not dependent on the nerve supply, it is the result of the neurosecretory 'eclosion hormone': a brain removed from the pupa and implanted into the abdomen of a decapitated pupa is wholly effective in evoking complete wing spreading. The suboesophageal ganglion is also necessary for wing spreading, but this seems to be a neural effect on movement. The hormone bursicon, set free from the abdominal nerve cord, will cause neither wing inflation nor wing folding; it has two important functions: it serves to plasticise the cuticle (as was shown by Cottrell in the blowfly) and it sets in motion the hardening of the cuticle which, in turn, apparently dictates by feedback to the central nervous system the timing of the wing-folding movements.

In brief the eclosion hormone, discovered by Truman a few years ago, not only initiates emergence, but also wing spreading, which is brought about by a central motor programme involving neural and hormonal stimuli and some degree of sensory feedback.

Plant conifers and lose water

from Peter D. Moore

A KNOWLEDGE of the rate of water loss from a forest is essential both for forest water budget calculations and in the management of water catchments. Many comparative studies have been undertaken to provide such information, on forest stands and on other vegetation types. Differences in water loss from various types of vegetation are explicable in terms of transpiration rates, in the effect of vegetation upon evaporation direct from the soil by providing insulating layers of varying thickness and spatial structure, and in the interception of precipitation and its subsequent evaporation directly from leaf surfaces.

The quantification of these parameters, however, is extremely difficult. One of the most useful approaches has been the development of detailed models which attempt to account for all of the microclimatic and spatial variables, each of which can be determined individually (for example, Monteith, *Symp. Soc. exp. Biol.*, **19**, 205, 1965). Various attempts have been made to compare water losses from deciduous and coniferous stands by observational rather than theoretical methods, but most of these have shown negligible differences during the summer months (for example Zahner, *Forest Sci.*, **1**, 258, 1955), no reliable data are available for longer periods covering the winter season, when evergreens may continue to transpire and also to intercept more rainfall than deciduous trees. Comparative data between forest stands on different sites will always be somewhat suspect because of possible differences in the soil water storage capacities, hence long term studies on a single site where deciduous trees have been replaced by conifers seems to be the most hopeful approach to this problem.

The results of just such a study have now been published by Swank and Douglass (*Science*, **185**, 857, 1974). Their study area is in the Appalachian Mountains of the south-western region of North Carolina, originally bearing oak–hickory forest. Streamflow studies were carried out on selected watersheds within the area over a period of ten years, which provided data sufficient for the prediction of stream flow under any given conditions of precipitation In 1956 a 16 hectare watershed was clear-felled and planted with eastern white pine (*Pinus strobus*) For the next six years the streamflow was greater than that predicted for the forest stand, in the initial period by as much as 15% Subsequently streamflow fell below predicted annual values and for the past 6 yr it has been between 15 and 20 cm (equivalent to 15–20%) below that expected from a hardwood stand on the same site

Monthly streamflow values over the course of a year are particularly interesting because they show that between June and October the monthly streamflow is less than 1 5 cm below the expected value, whereas in November, December, April and May streamflow is over 2 0 cm below expected Thus the additional losses of water from a conifer stand take place mainly in winter and spring, the possibility of this being the case had already been postulated by Penman (in *Forest Hydrology,* edit by W E Sopper and H W Lull, Pergamon Press, Oxford, 373, 1967) on the basis of very little observational evidence

The difference is likely to be caused by both the increased interception and the higher transpiration rates in the conifers in winter and spring The leaf area index of hardwoods in winter is low (<1) in comparison with white pine (9 9), which indicates the much larger surface area with pine available for the interception of precipitation

This provides one more good reason for caution in replacing hardwood forests with conifers, especially in water catchment sites

Field effect seen in glassy semiconductors

from Andrew Holmes-Siedle

AT the start of the solid-state age, Bardeen and colleagues attempted to modulate the conductivity of a slab of germanium by putting a metal plate near to the surface and applying a high electric field Unexpectedly, the conductivity of the slab did not change and, because of this, the idea of the 'field-effect transistor' lay dormant for over ten years while the reasons for failure were explored Meanwhile, in order to meet the urgent pressure for a working solid-state amplifier, the device makers (Sockley and colleagues) went on to develop the less elegant point-contact and diffused bipolar transistor principles

The field-induced 'accumulation' or 'inversion' of current carriers in the germanium did not take place as predicted because immobile charge was generated in surface states The sheet of charge 'screened' the interior of the semiconductor and prevented the required bending of energy bands Nowadays, surface states in crystalline semiconductors are under fair control and the metal-oxide–silicon field-effect transistor is a standard component in computer circuits It might now be said that we are at the start of the 'glassy solid state' age, it is perhaps a good omen that, despite a slow start, the field effect experiment can apparently be performed successfully in some glassy semiconductors This is fortunate because the penetration of a semiconductor by a field provides a probe of band structure which is particularly appropriate for the glassy semiconductors

Table 1 Estimated densities of localised states in chalcogenide glasses deduced from field-effect experiments

	Fritzsche	Egerton	Tick *et al*
Typical composition	$As_{35}Te_{28}S_{22}Ge_{15}$	$Te_{50}Si_{12}Ge_{10}As_{10}$	$Te_2As\ Si$
Form	Evaporated	Evaporated	Melted and drawn
Bulk states ($eV^{-1}\ cm^{-3}$)	6×10^{19}	10^{19}–10^{20}	3×10^{17}– 2×10^{19}
Surface states ($eV^{-1}\ cm^{-2}$)	not estimated	2×10^{13}	2×10^{12}– 3×10^{16}

Spear and le Comber (*J non-cryst Solids,* **8–10**, 727, 1972) gained some information on localised states within the band gap of amorphous silicon, but H Fritzsche failed to find any effect in the 'switching' materials, of chalcogenide composition, despite painstaking attempts (*Ann Rev Mater Sci*, **2**, 697, 1972) R F Egerton (*Appl Phys Lett*, **19**, 203, 1971) reported definite field effects which were, however, too small to be useful in determining the nature of the localised states Now, by special methods of preparation, Tick and Watson of Corning Research and Development labs and Hindley of the University of Birmingham (*J non-cryst Solids,* **13**, 229–242, 1974) have prepared samples which have lower apparent densities of localised states and which thus give a higher response to the field effect There is, therefore, some hope of using the field effect to determine the distribution with energy of the localised energy states in this very important group of materials

The fabrication technique used was unusual It is a fair assumption that the evaporated glass layers used by many investigators are more disordered than glasses cast and cooled from the melt But a field-effect experiment on a sizeable cast block of glass would be hopelessly swamped by the conduction currents in the bulk of the sample In order to make the bulk volume of the sample commensurate with the thin field-modulated region on the surface, Tick and co-workers used a thin filament of chalcogenide with an unusual form of surface passivation The filament was produced by melting and drawing down a sample of chalcogenide powder enclosed in a special silica glass jacket The result was a thin silica glass rod of diameter 7 mm with a very thin central filament of chalcogenide, of diameter only 0 01 mm When this was sliced, one could gain contact to the ends of the filament with probes while the glass disk contributed support for an annular metal field plate as well as providing surface passivation and mechanical strength For normal semiconductors and high fields, the surface space-charge regions are of the order of micrometres, so with these dimensions there is a chance that the field will influence a fair fraction of the filament volume

In fact, negative fields of the order of 10^6 V cm^{-1} modulated the bulk conduction by about 5%, though greater fields produced little further effect, this could be termed a saturating dependence on field Positive fields (metal positive) gave a much smaller effect The simple analysis which best fits the saturating characteristic is that of a density distribution of localised states which is uniform with energy all across the band gap The field dependence of current predicted for a sharply peaked distribution of localised states, such as that called for in the Davis–Mott model for chalcogenides (see for example Mott and Davis *Electronic Processes in Non-crystalline Materials,* Clarendon Press, Oxford, 1971) is of a very different form from that observed

The model proposed also postulates a significant density of surface states The figures for density are compared with the estimates made by Egerton and by Fritzsche in Table 1 The values given for certain samples in this latest work are seen to be considerably lower than in the earlier efforts—possibly a hopeful sign that we are learning better control of the preparation of glassy semiconductors

These results, while not constituting a definitive disproof of the commonly accepted peaked energy distribution of the Davis–Mott model show that the field-effect method is a tool which, with perseverance, may provide definitive evidence on this question

articles

Human reproduction and family planning: research strategies in developing countries

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The difficulties of providing family planning care on a world-wide scale have led to considerable emphasis on research in human reproduction How much of this research should, and can, be carried out in developing countries raises many issues in research administration

RESEARCH into human reproduction and family planning has expanded considerably in the past 15 years, and is now in progress in many institutions both in developed and developing countries Rarely, however, has sufficient effort gone into formulating appropriate national policies for such research

Developing countries raise some of the severest problems, frequently, esoteric research done in developed countries is repeated, while opportunities for local clinical investigations which are closer to the health needs of the countries are ignored The nature of research training may be partly to blame, trainees may be exposed to a technique rather than being provided with an approach to research and a broader background in reproduction The same incoherence is often found in the location of facilities new institutes are created where expansion of existing ones would have been sounder and more economic Funds are at times monopolised by small groups of scientists, and a contributory factor has been the multiplicity of funding sources, foreign ones often being the most important

The urgency of this research and the relatively ready availability of funds means that policy issues require serious consideration The World Health Organization recently convened a workshop for this purpose—research administrators from Argentina, India, Pakistan, Thailand, Turkey and the United States met to pool experiences Their problems can be discussed under the following heads

- Should research be done?
- How should priorities be determined?
- What administrative structure is necessary?

Should research be done?

Increasingly, policy makers are asking for clear orientation of research towards specific problems Stephen Toulmin, in *Encounter,* has described this process as " calling in the implied technological IOUs previously issued by the scientists" Policy makers also ask whether better use of existing knowledge and resources, or a simple increase in services would not better solve problems than would more research Do methods of fertility control now available (whether by drugs, devices or surgical procedures) not meet the needs of the great majority of couples? Is not the need now to make these methods more widely available?

We believe there is still a need for research The low initial acceptor rates and high discontinuation rates for present methods in almost all family planning programmes of developing countries are undoubtedly due in part to a lack of services, often, however, they are due to the wrong service approach At the same time contraceptive technology still leaves room for improvement Although more methods are now available, they remain relatively crude At times they are associated with undesirable side effects, and they often fail to meet the needs posed by differences in culture, education, and service-structure Moreover, many forms of contraception are still not available a male pill, a post-coital pill, a once-a-month pill, a vaccine against pregnancy and so on

Fertility regulation presents special problems for research techniques require an assessment well beyond that required for other therapeutic modalities because of their extremely wide, long term and frequently unsupervised use Further, expected users differ in genetic constitution and environment In most cases return of fertility must be allowed and methods are usually self selected, not prescribed Even conceding the need for more research, the role of developing countries in such research must still be clarified

- For established methods, dosages appropriate for women of a high level of nutrition may not be applicable Side effects of concern in the West may not manifest themselves, while new side effects may appear arising from disease and deficiency states in developing countries
- For new methods, developing countries should be involved at an early stage of the research to determine acceptability They must also be involved in testing at all stages of development so that safety and effectiveness data can be acquired rapidly for different populations
- As far as services are concerned, the argument that family planning programmes can go forward on a common sense basis, without any service research, does not stand scrutiny We have no precedent for providing health services on a continuing basis to most couples of reproductive age in a community Sensible studies and small experiments are needed on the major components of family planning activities in a local context information, education, and provision of clinical services For instance, inappropriate presentation of educational material may spoil an otherwise well-designed programme Local research is also essential in determining the right location of facilities, personnel, frequency and timing of clinics, schedules for follow-up care and provision of methods

How should priorities be determined?

Data are frequently lacking on how to identify specific problems, how to rate their relative importance, how to determine their susceptibility to solution through research, what manpower and facilities are needed, what is the potential pay-off, and what is the likely duration and cost On the other hand, fundamental questions of strategy have to be settled, such as the balance between goal-oriented and non-directed research, between research and research training, and between research in universities, government institutes and industry

The usual guidelines for setting priorities in biomedical research are the levels of unresolved morbidity and mortality, and indices of general welfare The same criteria hold here Initially, broad problem areas must be identified and ranked in importance pregnancy, labour, development, family planning and sterility Then specific topics for study and research approaches must be ranked For instance, should the emphasis be on development of new therapies or on service research?

The contribution of research to reducing morbidity and mortality has already been demonstrated in some fields, such as pregnancy and labour, beginnings have been made in research on the biology and health of the foetus and the foeto–maternal complex This research has generally been in developed countries Malnutrition and infectious diseases in developing countries are likely, however, to lead to great incidence of pathology and different forms of conditions associated with pregnancy, labour and foetal development

In ranking family planning research, account must be taken of indices both of morbidity and mortality and of 'welfare' Birth control prevents death and disease that result from unwanted pregnancy, short birth intervals, or illegally induced abortions, whilst the advantages of determining family size need little elaboration at the family level and will accord, in many communities, with population policies

Problems of local importance undoubtedly have first priority in developing countries The epidemiological, clinical, and service research required clearly cannot be carried out elsewhere Amongst such problems are the effects of hormonal contraceptives in women infected with *Schistosoma haematobium*, foetal development in communities with a high incidence of thalassaemia and prenatal care in protein-deficient pregnant women

More controversial are the priorities for problems that could be investigated in a developed country, for example, development of new contraceptives Ideally, as we have said, any new drug should be investigated in developing and developed countries simultaneously, using a common protocol This, however, demands highly specialised manpower and equipment Should scarce resources be expended on the earlier stages of development, such as those that precede testing on human subjects, for instance, identification of reproductive processes susceptible to regulation, synthesis or isolation of suitable chemicals, screening for effectiveness and toxicological studies in animals?

Several arguments can be made for not automatically excluding from developing countries any aspect of research needed to resolve important problems One is that it would make little sense and detract from the intellectual challenge if there were arbitrary cutoff points to research Another is that the whole range of scientific expertise must be available in a developing country, since scientists and institutions in developed countries are often not interested in the problems of the developing country An example is the lack of research on injectable contraceptives—a modality that meets many of the needs of developing countries An artificial limitation would further deprive the scientific community of talent and would preclude scientists in these countries from pursuing freely their choice Finally, university teaching is improved by the presence of an active research programme

Should public funds support research that is left entirely to the choice of the individual? A comparison of non-directed with directed research is difficult to make, and the level of funding for non-directed research cannot yet be determined on the basis of general principles

Feasibility of research on priority problems

A problem may be important and it may be seen to be amenable to solution through research, but it will have to be ranked in terms of the manpower and facilities that it would tie up Work can sometimes start immediately on a problem not of the highest priority, while a more important one must await the necessary resources A 10–20 yr plan, regularly reviewed, is needed

A representative ranking of research areas in many developing countries could take the following form First, the health rationale for family planning (for example, studies of effects on health of different patterns of family formation, identification of women particularly requiring family planning care because of their health status), second, clinical trials of contraceptives at the community level, third, the effects of widely-used contraceptives in the presence of conditions such as malnutrition, diseases, use of other drugs, fourth, the acceptability and service implications of different methods of contraception, fifth, sterility, and, sixth, the in-depth, pharmacological studies in humans of new contraceptives

Some developing countries may be able to start rapidly on the first item as they may already have manpower in the required disciplines such as obstetrics and gynaecology, maternal and child health, sociology, biostatistics Specialised technicians may need to be trained for field work, computer analysis, and clinical laboratory measurements This might take about three years If centred around one or two good investigators, a research nucleus would have been created which could then, if required, move into research projects in almost all of the priority areas

In contrast, item six demands manpower and facilities unlikely to be present in many countries Several years may be needed to train specialists with skills in steroid assays, peptide chemistry, radioisotope use, pharmacology, metabolic studies and so on Technicians are also essential as is the availability of specialised clinical facilities

One issue that repeatedly comes up is the proportion of funds to be spent on defined projects as opposed to longer-term more comprehensive 'institution-building' ones Assurance of financial support over a number of years is of importance to all investigators, but particularly so in the case of institution-building

A shortage of qualified manpower may make it difficult to bring together experts, other than the applicants themselves, to review research proposals for scientific merit, so foreign assistance may be necessary The low level of literacy in a population may pose problems in ensuring that 'informed consent' has been obtained

Should research training be restricted, for instance, to clinical trial methodology, or widely based in clinical pharmacology? The narrow approach quickly produces an investigator able to apply himself to an important problem, the more broadly educated scientist is potentially more versatile During his training, however, he may find his interests shifting away from clinical trials Western research training for scientists from developing countries has been problematical we may mention the 'brain drain' and the lack of orientation of the research training either to problems or conditions the scientist will encounter on his return home Failure to provide a research position for the investigator on completion of his training, to ensure attractive career opportunities regularly leads to loss of talent to the developed countries, some countries are now trying to remedy these shortcomings

Keeping up with rapid advances presents a technical and financial problem Airmail subscriptions to journals, access to computer retrieval of bibliographies, participation in international meetings go some way to help Less attention has been given to ensuring that research results rapidly reach policy-makers, administrators and service personnel in a form appropriate to their needs

What administrative structure is necessary?

The number of agencies with interests in research in this field is probably greater than in most other areas of biomedical research The concerns of such bodies as the Ministry of Health, the national Family Planning Authority, the Ministry of Education, the Medical Research Council (MRC), the Council for Science and Technology, the government offices responsible for the national drug industry, the national drug regulatory agency and so on, may be difficult to unite in a common policy It may be even harder to bring in the private drug industry whose orientation is primarily commercial Yet another factor is the aggressive attitude sometimes taken to family planning, including its research aspects, by foreign aid assistance agencies, whose priorities may not necessarily coincide with national ones

The national MRC sometimes offers a plausible focus it has experience in formulating biomedical research policy, could place research in reproduction and family planning within the overall context of the country's biomedical research programme, and could use its existing mechanisms for administration In many developing countries, however, there may not be an MRC capable of assuming these responsibilities, a ready focus may then exist in the Ministry of Education in whose institutions much of the research will be carried out anyway Another approach creates a body within the national family planning authority for the administration of research This should ensure a research policy directed to the problems encountered in the family planning programme and ensure rapid transmission of research results to field workers, unfortunately, such bodies may have little tradition of research, encounter resistance from older established ministries, and restrict themselves to a narrow and short term view of research

External aid has often been forthcoming, but on occasions not so much to support research efforts as to influence family planning policies, or even to gain access to subjects to test drugs under less stringent regulatory conditions than those prevailing in the industrialised countries The magnitude of the funds offered has at times led to this assistance being accepted in spite of the distortion it brought to nationally selected priorities Some countries now place rigid embargos on accepting assistance from any other than multilateral sources

The most neglected use of foreign assistance has been in the areas of policy and research organisation Few donors have wished to support activities that have little visibility and no quick returns, and few recipients have insisted on using research funds for this preliminary ground work

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Compositional variation in recent Icelandic tholeiites and the Kverkfjöll hot spot

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Geochemical studies of recent igneous rocks from Iceland lend support to the 'hot spot' theory There is a geographical variation in basaltic composition between Iceland and the submerged parts of the Reykjanes Ridge and Mid-Atlantic Ridge, which suggests that the single-source model for basalts of this region may be correct

THE puristic mantle plume hypothesis[1–3] holds that all ocean ridge volcanism is fed by the plumes The plate systems drift passively across the Earth's surface relative to the plumes, as shown by the universal symmetry of magnetic anomaly patterns about the ocean ridges Thus, the position of the ridges is not fixed by subcrustal agencies such as convection cells in the mantle Outside the mantle plumes there are no heat sources other than the plume overflows All the heat flow in the ocean bottom can be accounted for by the loss of heat from the cooling plates as they drift away from the ridges[4]

The single-source theory has been challenged by Schilling[5] on geochemical grounds, particularly with reference to Sr-isotope variation[6] We propose to show that geochemical variations in Iceland and the North Atlantic may be consistent with the one-source model, both as regards petrochemistry and isotope geochemistry

We have collected and analysed 182 samples that are considered to cover volcanic activity in the tholeiitic rift zones of Iceland[7] during the last 10^5 yr The geological formations represented are the 'grey dolerites' from the last interglacial, pillow lavas and breccias of the subglacial moberg (palagonite) formation, and postglacial lava flows The volumes of the individual lavas vary greatly monogenetic shield volcanoes range from 1–15 km^3, whereas lavas produced on fissures and isolated scoria cones are much smaller—generally less than 1 km^3

The samples have been divided into three groups on a geographical basis (Fig 1) representing the western rift zone (WRZ, 126 samples), the northern part of the eastern volcanic zone[8] (NEZ, 26 samples), and the Kverkfjoll area (30 samples), a relatively small area in the vicinity of the Kverkfjoll volcanic complex in central Iceland (K Albertson, unpublished)

The Kverkfjoll area is the hot spot of the North Atlantic Away from that area the maximum content of potassium in basalts on the volcanic rift zones decreases continuously with distance (Fig 2) The minimum K$_2$O content remains constant, at about 0 05–0 1%, in the Kverkfjoll area and probably along the entire adjoining ridges A pro-

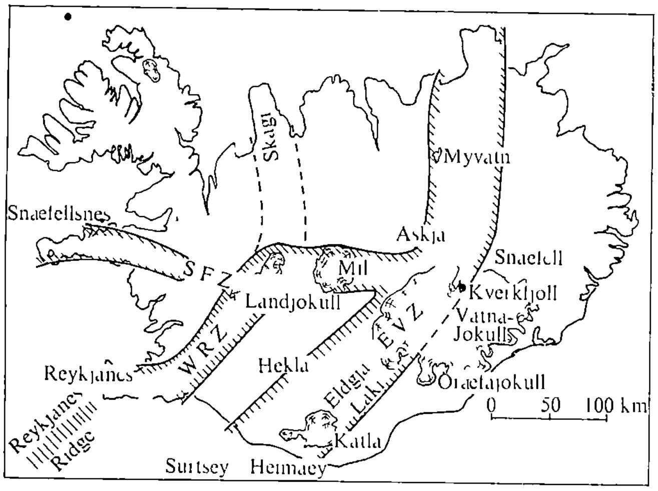

Fig. 1 Neovolcanic areas in Iceland The Snaefellsnes and the southern part of the eastern zones are alkaline in character, the others tholeiitic WRZ, Western Rift Zone, EVZ, Eastern Volcanic Zone, SFZ, Snaefellsnes Fracture Zone

nounced positive correlation exists between K_2O, TiO_2 and P_2O_5 whereas K_2O and Rb show insignificant covariation In central Iceland basalts with a high Rb content exist side by side with basalts highly depleted in Rb Away from the Kverkfjoll area the basalts show gradual depletion of Rb towards the ocean ridges together with increasingly narrower spreads in K_2O values At a distance of 500–600 km from central Iceland the K_2O content of the basalts reaches a value of 0 1%, with very little spread around that figure

The samples from the NEZ have been discussed in detail by Sigvaldason[8] A model was proposed to explain the large compositional spectrum in Icelandic basalts as compared with the narrow range observed in the bulk of ocean ridge basalts The analyses presented here corroborate those results In the model it is assumed that the Icelandic mantle plume feeds all volcanism in the North Atlantic The thermal gradient within the plume centre increases continuously to great depths in the mantle, providing a thermal environment for partial melting over a large depth range Conversely, as the plume overflow spreads below the crust along the volcanic rift zones in Iceland and the adjoining ridges, the thermal gradient goes through a temperature maximum at which partial melting occurs With increasing distance from the plume centre, the thermal maximum becomes better defined at a depth which happens to be appropriate for the formation of ocean tholeiites As that represents relatively low-pressure partial melting, all chemical types formed by partial melting at higher pressure gradually disappear (Fig 2) Thus, two processes are in evidence partial melting at varying depths, and progressive depletion of dispersed elements at the source

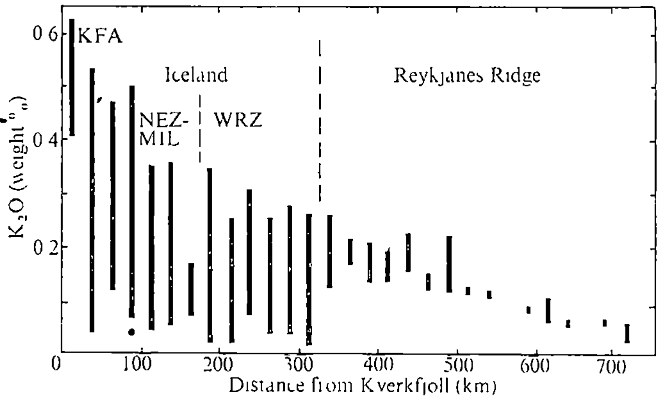

Fig 2 Range of K_2O values plotted against linear distance from Kverfjoll, central Iceland Each column represents the range obtained in a 25 km sector KFA, Kverfjoll area, NEZ, northern part of Eastern Volcanic Zone, MIL, Mid Iceland, WRZ, Western Rift Zone, (see Fig 1)

The Icelandic tholeiites span almost the entire spectrum of observed compositions of oceanic tholeiite To illustrate this is a plot of MgO against iron (Fig 3) compares the Icelandic tholeiites to analyses of oceanic tholeiites[9–11] In spite of this great compositional span, however, the bulk of the Icelandic analyses tends to fall on the iron-rich side of the oceanic analyses This is in keeping with the fact that, as regards volume and frequency, Icelandic tholeiites are distinguished by high alkalis and iron magnesium ratios[12] relative to oceanic basalts The Juan de Fuca[11] analyses resemble the Icelandic suite most (Fig 3), which is in accord with the idea that the Juan de Fuca ridge is the site of a mantle plume[2]

Within the Icelandic group the enrichment of iron and potassium, together with other related chemical variations, seems to bear an inverse relationship to lava volume[8] This effect could be attributed to fractional crystallisation[11] The Ti concentration, however, which has been shown to be sensitive to pressure[13], shows close positive covariation with K, and could be explained equally well by a degree of partial melting (compare with Fig 2) The basaltic liquids may have formed over a relatively large depth range in the mantle Frequent eruptions of small batches of lavas which have undergone only a low degree of partial melting, and which have been derived from a considerable depth, result in large total volumes of high alkali, high iron/magnesium tholeiites, relative to low potassium tholeiites which are produced at shallower depths by a higher degree

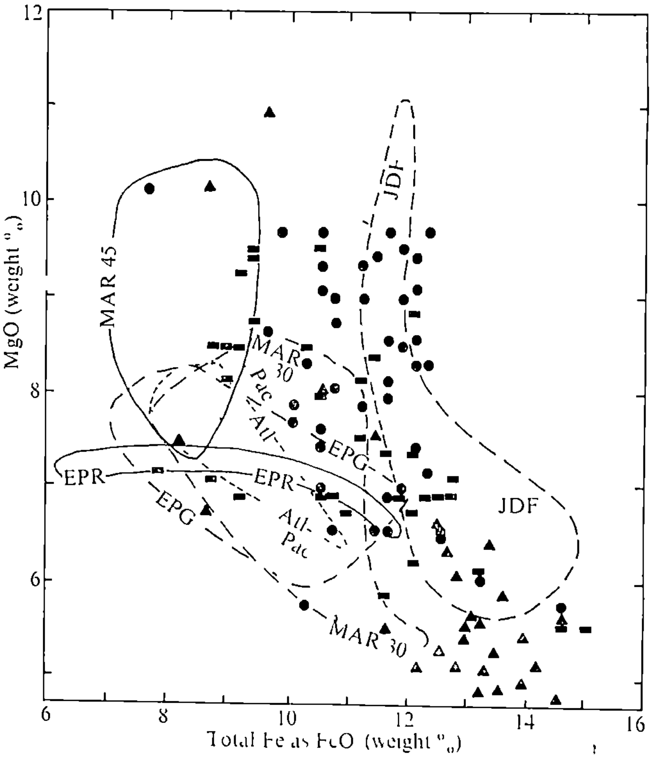

Fig 3 MgO plotted against total iron in the samples, showing the relatively iron rich nature of Icelandic tholeiites The range of values observed in oceanic tholeiites is shown for comparison Atl, Atlantic Ocean[9], Pac, Pacific Ocean[9], EPR, East Pacific Rise[11], EPG, Gorda ridge (41° N)[11], MAR 45°, Mid-Atlantic Ridge (45° N)[11], MAR 30°, Mid Atlantic Ridge (30°N)[11], JDF, Juan de Fuca Ridge (44°N)[11], ▲, Kverkfjoll Area, ■, North-eastern Zone, ●, Western Rift Zone

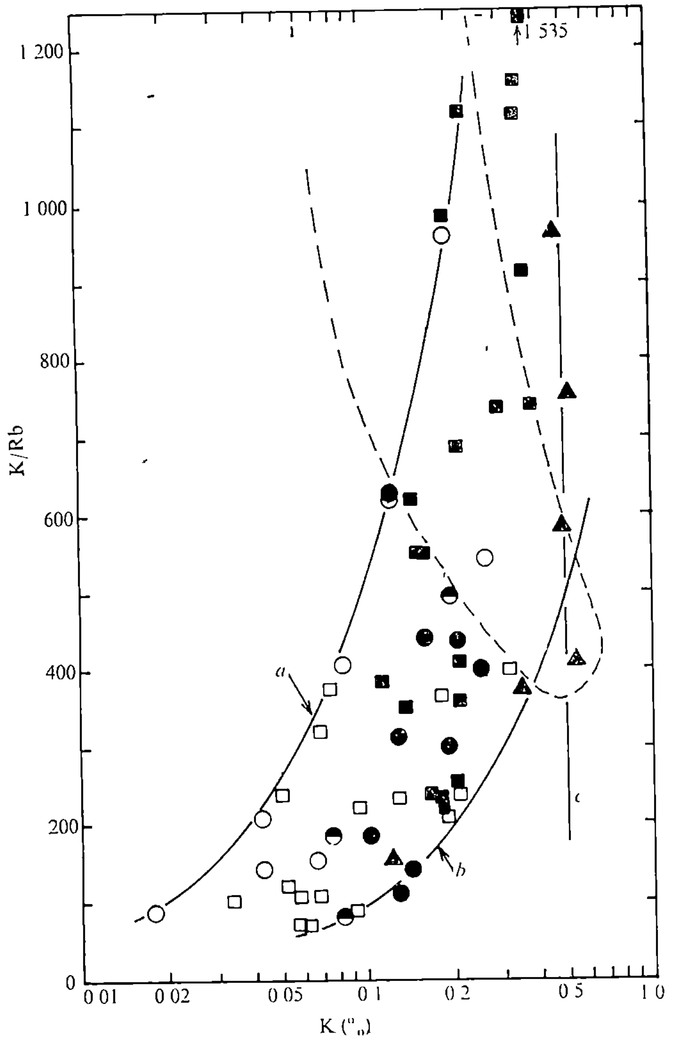

Fig. 4 The K/Rb ratio plotted against log K. Lower part of the field of oceanic tholeiites[11,16] enclosed by dashed line. *a*, Rb=2 p.p.m.; *b*, Rb=10 p.p.m.; *c*, K=0.5%; □, NEZ shield volcanoes and table mountains; ○, WRZ shield volcanoes and table mountains; ■, NEZ fissures and minor eruptions; ●, WRZ fissures and minor eruptions; △, KFA fissures and minor eruptions. The Rb values were obtained by XRF analysis. Although this has a distinct disadvantage at low Rb values this was overcome partially by adding a fixed amount of 20 p.p.m. Rb to the samples. The precision is considered to be better than ± 1 p.p.m.

of partial melting. The volume relationships between products of partial melting of high and low degree in Iceland are, therefore, entirely different from those of the ocean ridges. Along the Reykjanes ridge, in a direction away from Iceland, the low degree partial melting products disappear, giving an impression of an increase in the degree of partial melting at the same time as volcanic productivity decreases. This seems to explain adequately the apparent decrease in light rare earth elements south of Iceland[5].

Figure 4 shows the K/Rb ratio plotted against K for some of the samples, with the field of ocean ridge basalts[11,16] shown for comparison.

Several features of the diagram are worthy of discussion. The K/Rb values are generally lower than previously observed for tholeiites[11]. Analyses of plagioclase separates from porphyritic basalts show K/Rb ratios in the range 50–300. There is no consistent relationship between the K/Rb ratios of phenocrysts and groundmass. Furthermore, REE analyses of Icelandic tholeiites[14,15] do not show Eu anomalies, suggesting that there has been no plagioclase differentiation. These low ratios, therefore, may be derived from the mantle and could indicate a higher content of Rb (phlogopite for example) in the plume than has been assumed previously[17].

The K/Rb–K field is defined by three curves (Fig. 4): its low K side is bounded by the curve Rb=2 p.p.m., whereas for the high K side, at K/Rb ratios below 400, the curve Rb=10 applies, and for higher values the line K=0.5% (Fig. 4). The values for the shield volcanoes tend to fall on the diagram to the left of those of other type of volcanoes. As a group the shield volcanoes show the lowest K and Rb values in Iceland even if the K content may be higher, and the Rb content is much higher, than in the depleted ocean ridge basalts. The voluminous shield eruptions occurred in the specific volcano-tectonic environment found during the isostatic rebound of Iceland immediately after the Pleistocene glaciation. The prevailing conditions of pressure and temperature were suddenly disturbed towards lower pressures, resulting in the melting of large batches of mantle material that had already gone through an earlier stage of melting. They are the most depleted basalts in Iceland.

Schilling[5], chiefly on the basis of Sr isotopes[6], has advocated two primary magma sources in the North Atlantic. Magmas derived from the Icelandic plume have high (~ 0.7032) ^{87}Sr/^{88}Sr ratios[6,15,18], show an undepleted REE pattern, and have generally high contents of K, Ti, P, and high Fe/Mg ratios. The oceanic source yields lavas with a much more depleted chemistry and lower (0.7026–0.7028) Sr isotope ratios[6]. The gradient in composition, from Iceland along the ridge, results, according to that theory, from the mixing of the two magmas. The observed variation in Sr isotopes can possibly be accounted for in terms of the single source model. At the depth at which magma segregation is supposed to take place the upper mantle consists of olivine, two pyroxenes, plagioclase and minor phases, including phlogopite[19] and amphibole[20,21]. The K and Rb enrichment of the basalts in central Iceland results from the important contribution of phlogopite to the early partial melts of the plume. Phlogopite contains about 7% K, 250 p.p.m. Rb, and 18 p.p.m. Sr (ref. 17). The other components in the upper mantle contain between 1 p.p.m. and 0.1 p.p.m. Rb. The high Rb content of the phlogopite results in an increased ^{87}Sr/^{86}Sr ratio relative to the rest of the mantle. Using the values of Rb and Sr already given for phlogopite the ^{87}Sr/^{86}Sr ratio would increase by 7×10^{-5} every million years. Conversely, the highest Rb/Sr ratios observed in Iceland tholeiites increase by 2×10^{-6} every million years, whereas the Sr isotopic ratio of the depleted ocean tholeiites has very little capacity for change with time and probably reflects that of the bulk of the upper mantle.

The implication of possible isotope disequilibrium between mineral phases in the upper mantle may be far reaching as regards the interpretation of isotopes in partial melts. As long as a given mineral assemblage remains stable, however, each mineral may develop its own isotope ratio—which is the basis for the isochron method of radiometric dating[22]. Unless recrystallisation occurs there seems little impetus for isotope re-equilibration between phlogopite and the mantle phases with a low Sr content. In general, because Sr is produced in phlogopite by radioactive decay it should tend to migrate one way only—away from the phlogopite.

There is, as yet, insufficient documentation of the relationship between precisely measured Sr isotope ratios and the petrochemistry of Icelandic rocks. Preliminary work[6,15] indicates, however, that, in general, rocks from the alkalic provinces in Iceland possess slightly higher Sr isotope ratios than the tholeiites. That is consistent with their supposed nature as lower degree partial melts[19].

On the basis of the available data we therefore conclude that there is no well established geochemical evidence to contradict the single-source model for the North Atlantic There is a continuous gradational variation between basalts erupted in Iceland and basalts on the submerged part of the Reykjanes and Mid-Atlantic Ridges This compositional gradient probably results from the combination of a changing environment of pressure and temperature in an upwelling and radially spreading mantle plume system, and from a progressive depletion in the low temperature melting fraction at the source

Note added in proof Unknown to us at the time of writing, similar ideas on isotope disequilibrium in the mantle have been published[23]

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Climatic significance of deuterium abundance in growth rings of *Picea*

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Deuterium variations in growth rings of a Picea *from southern Germany are essentially a function of annual air temperature Short term isotope variations are partly influenced by changes of relative humidity, but it is the long term annual temperature fluctuations which are mainly responsible for the deuterium variations in the rings Long term deuterium variations in* Picea *record climatic changes of the past and also reflect the deuterium content of the annual precipitation*

NATURAL, stable isotopes are used more and more frequently in the field of palaeoclimatic research Apart from ^{18}O thermometry[1,2] on sea cores, measurement of the deuterium content of plants opens new aspects for palaeoclimatic investigations

The basic idea of the deuterium method is that the natural deuterium content of chemically bound hydrogen in plants is correlated with that in precipitation[3] As the deuterium content of precipitation in middle and high latitudes depends mainly on the climate and decreases with annual mean temperature[4], the deuterium content in plants is closely related to climate[5], and tree rings should therefore bear information on past climates

Sample preparation and mass spectrometric deuterium determinations have already been described[3] The overall measuring accuracy is ± 2‰ and the results are reported in per mille deviation from Standard Mean Ocean Water (SMOW)[6]

During evapotranspiration of the leaves, complicated isotopic interactions occur between atmospheric vapour and water which is replenished from the soil The hydrogen component of the water is transformed by photochemical processes into woody matter and so evapotranspiration is a process which is important. in the development of the deuterium content of plant material

Isotopic equilibria in these interactions can be described mathematically[7]

$$\delta_l = \delta_s\,(1-h)+h\delta_v+\varepsilon_p+\varepsilon_k\,(1-h), \qquad (1)$$

where δ_l is the deuterium content of the leaf sap, δ_s that of soil water, and δ_v that of atmospheric vapour ε_p is the equilibrium separation factor which increases with decreasing temperature, ε_k is the kinetic separation factor, and h the relative humidity This formula describes the isotopic processes during evapotranspiration in leaves to a good degree of approximation[8]

The average deuterium concentration in leaves from a *Picea* was calculated for the period 1881–1970 For the calculations I used meteorological data from the Hohenpeissenberg Observatory (unpublished information and ref 9), which is 16 km east of Sachsenried, the place where the tree grew Measurements of the deuterium content of precipitation and ground water of this area indicated that $\delta_s \approx 70‰$ Because atmospheric vapour in Central Europe, contains on average, 75‰ less deuterium than does precipitation $\delta_v \approx \delta_s - 75$ (ref 10) ε_p is temperature dependent and is +93‰ at 15° C (ref 11), and ε_k (= 16‰) is independent of temperature The relative humidity in the area around Sachsenried is about 75‰, and

Table 1 Correlation coefficients between the deuterium content of *Picea* and meteorological and other parameters

	1785–1910	1910–1970	1785–1970	1785–1970
		5-yr means		10–20-yr means
$r_2\sigma$	0 4	0 6	0 33	0 54
Summer temperature	0 035	0 52	0 04	0 41
Annual temperature	0 51	0 74	0 47	0 83
Humidity	—	0 47*	0 056*	0.34†
Leaf sap	—	0 57	0 43*	—
δ_{18} ice	—	—	—	0 61

* $r_2\sigma$ = 0 49, † $r_2\sigma$ = 0 7 (beginning in 1881)

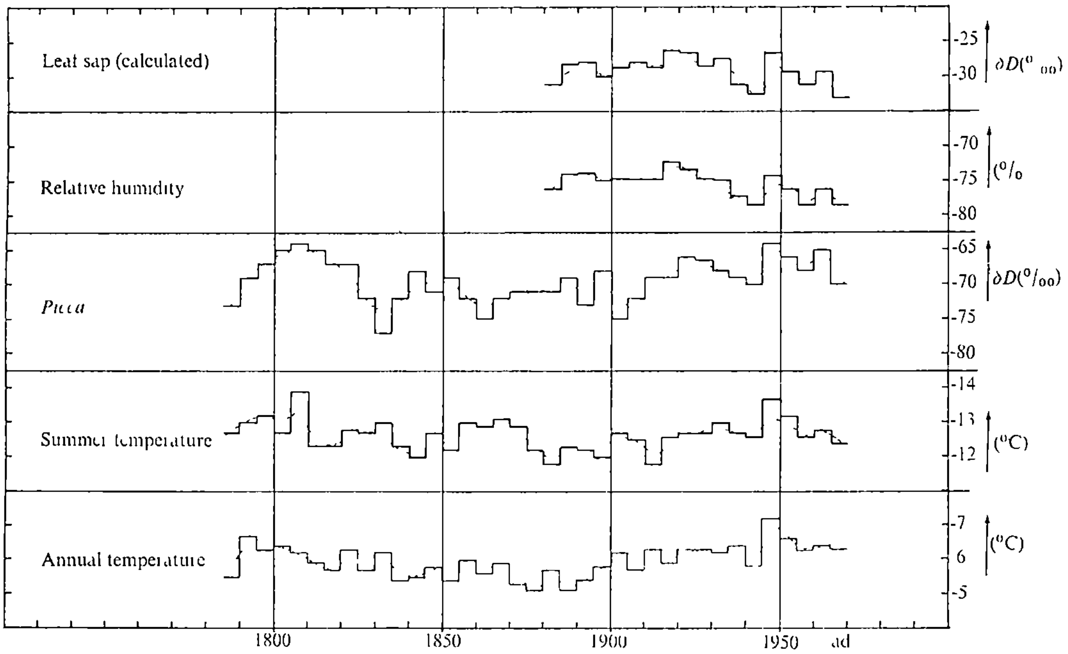

Fig. 1 Comparison between summer temperature (mean May–September), annual mean temperature, deuterium content of *Picea*, relative humidity (mean May–September) and the calculated deuterium content of the leaf sap all in the 5-yr means Dashed lines indicate the trends in the histograms

so equation (1) shows that the influence of the atmospheric vapour is more significant than the influence of the water which the plants take up

Implications of a deuterium content

From equation (1) it follows that the deuterium content should depend essentially upon the temperature and relative humidity during the growing season In order to test this conclusion the deuterium content of growth rings of a *Picea* from Sachsenried (46° 50′N, 10° 50′E), with an age of about 200 yr was determined For the period 1910–1970 each ring was analysed twice for deuterium to diminish any errors which may have arisen from the analysis of an incomplete ring Growth rings formed between 1785–1910 were analysed In each case a block of rings formed in five consecutive years was analysed and care was taken to burn the same amount of wood from each block, so that a weighted mean was not obtained

The deuterium content of each block has been compared with the summer temperature (mean May–September), the annual mean temperature, the relative humidity (mean May–September) (Fig 1), and the calculated deuterium concentration in the sap of leaves (for the same summer period) using equation (1) In order to be able to compare the results for the various possible combinations in a statistically meaningful manner the correlation coefficients for the various periods with different measuring accuracies have been calculated (Table 1) The maximum error of the correlation coefficient for the 2σ limit is given as $r_{2\sigma}$ in Table 1

In the 5-yr mean, there is a reasonable correlation between the deuterium content and the summer temperature ($r = 0\ 52$, $r_{2\sigma} = 0\ 6$) in contrast to the poor correlation with mean annual values In particular, the deuterium trend is distinctly indicated by the trend of the temperature curve A perfect correlation cannot be expected in any case, because the meteorological parameters are averaged continuously over five months—that is not the case for the deuterium content of wood On overcast days there is hardly any assimilation because of insufficient intensity of solar radiation Furthermore, the humidity still shows some fluctuations in the 5-yr mean, so some degree of influence of the humidity on the deuterium content is evident On the long term mean the humidity variations level off and the trend of the deuterium curve follows more perfectly that of the temperature curve (Fig 1)

The measuring accuracy is best for the period 1910–1970,

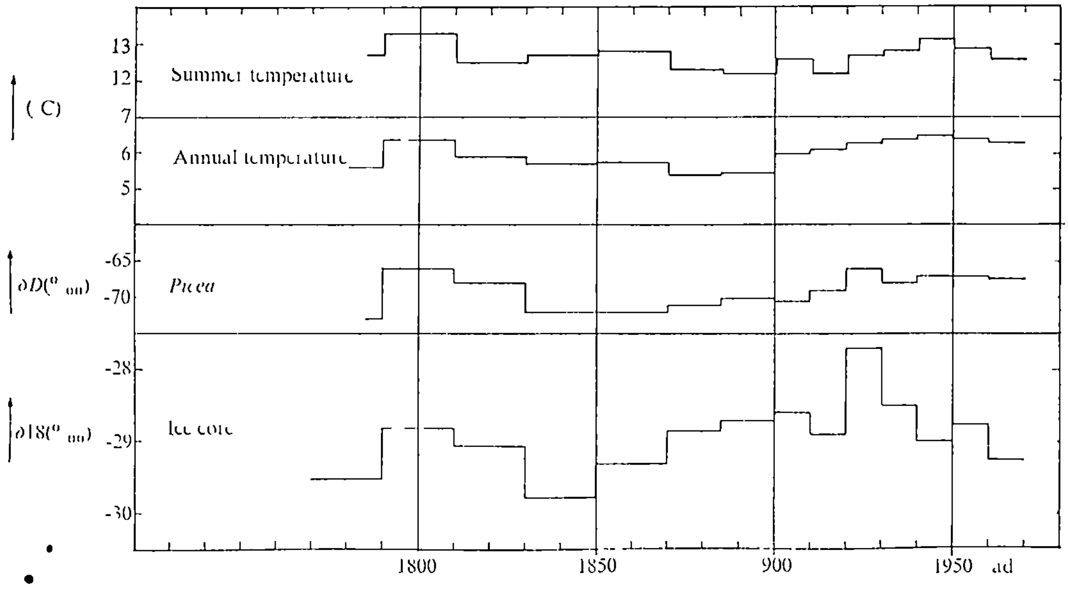

Fig 2 Comparison between the ^{18}O content of an ice core from Camp Century on Greenland and the deuterium content of the growth rings of a *Picea* from Sachsenried The upper graphs show the summer and the annual mean temperatures from the Hohenpeissenberg, Meteorological Observatory near Sachsenried

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because the average of 10 single measurements is used In the period 1785–1910 the accuracy is poorer because only two single analyses are averaged (Table 1) and the time scale for the older year rings becomes increasingly inaccurate because some rings are missing

The deuterium variation of the leaf sap is smaller than that of the growth rings Presumably, that is because the calculation is based on a constant correlation between the deuterium content of atmospheric vapour and the atmospheric temperature (3‰ ° C^{-1}) for Central Europe Such a relationship, however, is an idealisation of the natural conditions, because at any one temperature, depending on the meteorological conditions, substantial variations in the deuterium content of atmospheric vapour can still occur

The growing period comprises hardly half a year, and so it must be considered whether the climatic processes during that period really reflect, to any degree, the general climatic trend of the entire year A comparison between summer and annual temperatures shows that similar trends do, in fact, occur The low temperatures of the 19th century, which caused a considerable advance of the mountain glaciers, in temperature latitudes, are indicated clearly In addition to that, a comparison of the correlation coefficients for summer and annual temperatures (Table 1—5-yr means) shows that the annual temperature correlates much better with the deuterium content of the growth rings (r = 0 47, $r_{2\sigma}$ = 0 33) than does the summer temperature (r = 0 04, $r_{2\sigma}$ = 0 33) That observation is further supported by the comparison in Fig 2 In the lower graphs the deuterium content of *Picea* is compared with the ^{18}O result from an ice core from Camp Century on the Greenland ice sheet[12] There is a good relationship, although the ice reflects the isotopic composition of the annual precipitation (r = 0 61, $r_{2\sigma}$ = 0 54) On the long term mean the relationship between the deuterium content again seems better with the annual termperature (r = 0 83, $r_{2\sigma}$ = 0 54) than with the summer temperature (r = 0 41, $r_{2\sigma}$ = 0 54) I therefore conclude that the deuterium concentration in the atmospheric vapour is a function of the climatic history of the whole year and influences the deuterium content of the leaf sap so that, even though only a relatively short growth period is measured, the results reflect throughout a whole year That is important for all palaeoclimatic investigations which use deuterium variations in biogenic material Finally, as two localities thousands of kilometres apart show similar isotopic trends, it follows that essentially global climatic changes must be responsible for the deuterium variations in *Picea*

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Properties of hybrids between *Salmonella* phage P22 and coliphage λ

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The temperate bacteriophages P22 of Salmonella typhimurium *and* λ *of* Escherichia coli *form viable hybrids in which the immunity, early control and DNA replication genes of P22 are substituted for the analogous* λ *genes The specificity of the early control and DNA replication genes differs between P22 and* λ, *the immunity of the hybrid is identical with that of the lambdoid coliphage 21 Implications for the evolution of viruses are discussed*

The temperate bacteriophages P22 of *Salmonella typhimurium* and λ of *Escherichia coli* are very similar in functional organisation[1,2] In particular, the regions concerned with control, recombination, integration, DNA replication and lysis functions of both phages are arranged in the same order on their genetic maps (Fig 1) The phages share DNA sequence homology, as measured by DNA–DNA hybridisation[3], most of which occurs in the right arm of λ DNA[4], where the functions listed above are located These similarities are counterbalanced by profound differences between the two phages (1) P22 DNA is circularly permuted and terminally repetitious, whereas λ DNA is not permuted and has single-stranded cohesive ends[5,6], (2) The P22 genetic map is circular, whereas the map of λ is linear[7,8], (3) P22 particles have only a short baseplate structure whereas λ has an elongated tail, (4) P22 adsorbs only to *Salmonella typhimurium* whereas λ adsorbs only to *E coli*, (5) Control of lysogeny differs P22 has two regions essential for immunity and repression whereas λ has but one[11,12], (6) P22 lysogens exclude superinfecting phages in several ways beyond the immunity and repression system whereas λ lysogens display solely the immunity provided by the single repressor[13,14]

These considerations led us to inquire whether hybrid phages containing DNA from both P22 and λ could be constructed, and, if so, which blocks of P22 genes were interchangeable with analogous blocks from λ We show here that P22 DNA can be substituted in the right arm of λ to yield hybrid phages with distinctive properties The construction of P22/λ hybrid phages was accomplished independently by a different method by Gemski *et al* [15] Studies

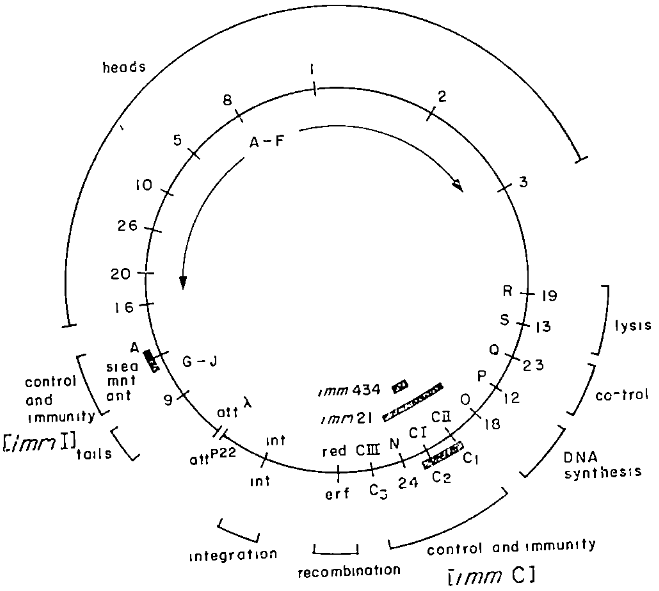


Fig 1 Comparison of the genetic maps of P22 and λ Inside the map are the λ gene designations, outside are the P22 gene designations One-to one correspondence means great similiarity in mutant phenotype The heavy bars outside indicate *imm*I and *imm*C, the heavy bars inside indicate the extent of non-homology between λ and λ*imm*21 and λ*imm*-434 The map is redrawn from Botstein *et al*[1]

with these hybrid phages indicate that P22 and the *E coli* lambdoid phage 21 (ref 16) have repressors and operators with apparently identical specificity, indicating common ancestry, on the other hand, the DNA replication and early gene control systems of P22 and the lambdoid phages are analogous but have significantly different properties

The cross

Phage P22 does not adsorb to *E coli* and phage λ does not adsorb to *S typhimurium* To get both phage DNAs into the same cytoplasm so that they might recombine, we took advantage of the ability of *E coli* episomes to be transferred between the two species of bacteria Roth and Hoppe[17] showed that the $F'lac^+pro^+$ episome of *E coli* carries a prophage attachment site for phage P22 Consequently, P22 was inserted into this episome by infection of an $F'lac^+pro^+$ *Salmonella* strain which was deleted for the P22 prophage attachment site on the chromosome The P22 carried on this episome has a temperature-sensitive repressor (*c*2 gene product), hence, the prophage is induced at high temperature in *Salmonella* strains The $F'lac^+pro^+str^s$ (P22*c*2-*ts*) *Salmonella* strain was mated with an *E coli* lac^-str^r strain, and *E coli* lac^+str^r recombinants selected Most of these recombinants were temperature-sensitive for growth and produced 20–50 P22 phage per cell at high temperature Therefore, it appears that all host components required for P22 growth are present in wild type *E coli* In contrast, it was found that λ^+ will not grow under similar circumstances in *S typhimurium*[18]

The hybrid phages described here are derived from an *E coli* lysogen-carrying P22*c*2*ts*30 prophage, when this lysogenic strain was infected with a marked λ (λ *c*I60) and the culture was shifted to 41° C a small yield of λ and P22 (about 20 of each) was produced Among the progeny were recombinants (about 0 01%) with the host range of λ (that is, adsorption to *E coli*), but which had acquired a new immunity (that is, growth in *E coli* lysogenic for λ) In a parallel infection, nonlysogenic but otherwise identical $F'lac^+pro^+$ *E coli* strain was infected with λ *c*I60 at 41° C, no phages able to grow on *E coli* lysogenic for λ were obtained

Structure of λ*imm*P22

(*a*) The immunity region derives from P22 Since the hybrid phages were selected for their ability to grow on a λ lysogen of *E coli*, it was expected that the hybrids had a new immunity, presumably from the P22 prophage This idea was confirmed by several observations (1) The hybrid phages all have a temperature-sensitive clear plaque morphology, unlike that of the λ parent, but like that of the P22 prophage As expected if the *ts*-clear mutation affects the P22 *c*2 gene, *E coli* lysogens carrying these hybrids as prophages are induced when shifted to 41° C (2) λ phages grow on the *E coli* strain lysogenic for P22 The hybrid phages do not grow on these lysogens, indicating that they have immunity of P22 The hybrid phages grow normally on the $F'lac^+pro^+$ *E coli* strain which does not carry a P22 prophage (Table 3) (3) In other experiments, hybrids were constructed between λ and P22 prophages which carry *amber* or *ts* mutations in the genes near the *c*2 gene, in these cases, the hybrid phages inherit the *amber* or *ts* phenotype (S Hilliker, unpublished work)

These bybrid phages—obtained from the cross of λ*c*I60 with P22*c*2*ts*30—are called λ*imm*P22 by analogy with λ*imm*434 and λ*imm*21, λ hybrids with the immunity specificity of the lambdoid phages 434 and 21 (refs 19 and 20)

(*b*) Tail genes derive from λ Since the hybrid phages can grow on *E coli* strains, the genes determining host range must be inherited from the λ parent As anticipated, the hybrid phages are as sensitive as λ wild type to antibodies raised against λ wild type phage particles Furthermore, mutants of *E coli* lacking the λ receptors for adsorbtion are also resistant to the λ*imm*P22 hybrids (data not shown) λ*imm*P22 hybrids, therefore, have tail genes from λ

(*c*) Prophage insertion specificity derives from λ The hybrid phages can integrate into an *F′gal att*λ episome and be tranferred into another strain by conjugation λ*imm*P22 hybrids, therefore, have the insertion specificity (attachment site and *int* gene) of λ (S Hilliker, unpublished work)

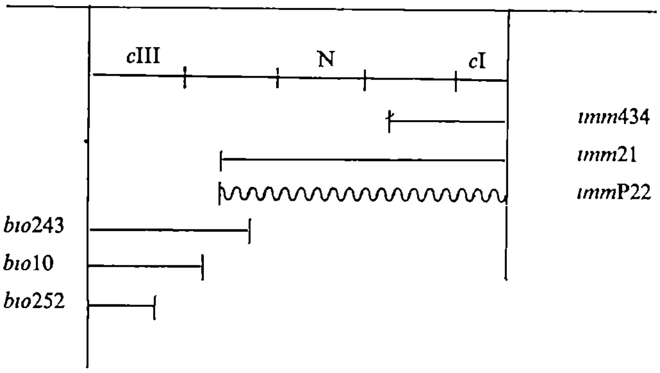
(*d*) The source of other genes in the hybrids is as follows Marker rescue tests were done by plating λ *amber* mutants on su^- (nonpermissive) lysogens of λ*imm*P22 hybrids For comparison, phage were also plated on lysogens of λ*imm*434 (known to substitute none of the essential λ genes tested) and λ*imm*21 (known to substitute only gene *N*)[21] Recombinants with immunity of λ and wild type gene function

Table 1 Efficiency of plating (marker rescue experiment)

	No prophage	λ*imm*434	Lysogen λ*imm*21	λ*imm*P22
λ*Bam*1	−−	++	++	++
λ*Jam*6	−−	++	++	++
λ*Nam*7	2×10^{-7}	1×10^{-5}	3×10^{-7}	4×10^{-7}
λ*Nam*53	1×10^{-6}	2×10^{-4}	1×10^{-6}	1×10^{-6}
λ*Oam*29	1×10^{-6}	5×10^{-3}	1×10^{-3}	2×10^{-6}
λ*Pam*3	3×10^{-8}	4×10^{-1}	1×10^{-2}	1×10^{-7}
λ*Pam*80	5×10^{-7}	2×10^{-1}	7×10^{-3}	7×10^{-7}
λ*Qam*73*Sam*7	$<10^{-8}$	3×10^{-2}	2×10^{-2}	8×10^{-3}
λ*Sam*7	−−	++	++	++
*Ram*5	−−	++	++	++

Lysogens were constructed in strain 594 which is strongly nonpermissive for *amber* mutants Phage were plated either for efficiency (numbers) or as a serial-dilution spot test Plates were incubated at 30° C since λ*imm*P22 carries a c_2ts allele λ*imm*21 and λ*imm*434 were described by Herskowitz and Signer[21]

Table 2 Lefthand endpoint of nonhomology region



Frequency of recombinants

	immP22	λimm21	λimm434
λ*bio*243	$<2\times10^{-7}$	$<2\times10^{-5}$	2×10^{-2}
λ*bio*10	2×10^{-3}	4×10^{-4}	—
λ*bio*252	1×10^{-2}	6×10^{-3}	

Crosses were done as standard λ crosses without ultraviolet-irradiation as described by Signer and Weil[33] Recombinants were scored by their ability to grow on *E coli* lysogenic both for P2 (which selects *bio*) and λ (which selects against *imm*λ) None of the parental phages grown in parallel produced progeny capable of growth on the test dilysogen at a frequency in excess of 2×10^{-5} In the diagram, solid line indicates λ DNA replaced

were scored Table 1 shows that functional alleles of mutations in λ genes *Q, S, R, B* and *J* can be rescued, whereas the functional alleles of mutations in λ genes *N, O* and *P* cannot

The appearance of nondefective *imm*λ recombinants in the cases of *Q, S, R, B* and *J* mutants indicates that the λ*imm*P22 prophage contains either (1) the wild type allele from its λ parent, or (2) an analogous and functionally compatible allele from the P22 parent which is separable from *imm*P22

Failure to find functional *imm*λ recombinants in the cases of *N, O* and *P* mutants indicates that the hybrid phage does not carry these genes from the λ parent, and that the analogous P22 genes in the hybrid phage cannot be separated from the P22 immunity region of the hybrid phage

These results suggest that in λ*imm*P22 the immunity region of λ has been replaced by an analogous region from phage P22 (Fig 1) The region of nonhomology (or non-compatible function) between λ*imm*P22 and λ in the hybrid apparently includes genes *N* to *P* on the λ genetic map The analogous region in P22 (*imm*C and its flanking genes) has been found to contain genes with functions analogous to those of λ (gene control and DNA replication in addition to immunity specificity)[1,22,23]

We have performed additional crosses to determine in detail the lefthand endpoint of the nonhomology between phages λ*imm*P22 and λ λ*bio*10*imm*P22 but not λ*bio*243-*imm*P22 recombinants can be formed, indicating that the region of nonhomology extends to between the endpoints of these deletions Table 2 shows that the lefthand endpoint of nonhomology of phage λ*imm*21 falls within same region Since the *imm*21 substitution replaces the λ *N* gene, these crosses provide independent evidence that the *imm*P22 substitution also replaces λ gene *N*

Functional comparisons of λ/P22 hybrids

(a) λ*imm*P22 has the same immunity specificity as λ*imm*21 λ*imm*P22 hybrids establish and maintain lysogeny normally, less than 1% of the cells in a lysogenic culture are cured These lysogens are immune to superinfecting phage of the same genotype We tested various lambdoid phages for the ability to grow in λ*imm*P22 lysogens We also tested the ability of λ*imm*P22 to grow in lysogens of these lambdoid phages As Table 3 shows, λ*imm*P22 grows normally in all lysogens except those carrying prophages with the immunity of phage 21 or P22 Furthermore, λ*imm*P22 lysogens are immune to those phages carrying the immunity region of phage 21 or P22 This result implies that the *imm*C repressor and operators of phage P22 are functionally equivalent to the repressor and operators of phage 21 and different from those of all other lambdoid phages tested It should be emphasised that the *imm*C region of P22 is sufficient to control the hybrid phage λ*imm*P22, even though two immunity regions (*imm*I and *imm*C) control wild type P22 This result means that *imm*C is probably the primary regulator of repression and immunity in P22

(b) We have obtained λ/P22 hybrids with substitutions of regions other than immunity To construct hybrids different from λ*imm*P22, the *F′lac*⁺*pro*⁺(P22) strain was infected with various defective λ mutants For example, this strain was infected with λ carrying *amber* mutations in both genes *Q* and *S* *amber*⁺ recombinants with the host range of λ were selected by plating the progeny phage on *su*⁻ nonlysogenic *E coli*, recombinants were obtained at frequencies between 10^{-3} and 10^{-4} These recombinants have less DNA than the Q^-S^- parent, as judged from their ability to grow in the presence of chelating agents[25] Most of these phages were able to grow in λ*imm*P22 or λ*imm*21 but not in λ lysogens, indicating that they had retained the immunity specificity of the λ parent If these hybrid phages were formed by the minimal recombination event (a double crossover), they should carry the P22 region analogous to the *Q*–*S* region of λ Since both phages in this region confer the specificity of late gene expression[1 25], these hybrids should contain the head and tail genes of λ and the late gene control region from P22 Preliminary studies (P Toothman and

Table 3 Immunity of λ*immP*22

	F′*lac* (P22)	F′*lac*	No prophage	λ*immP*22	λ*imm*21 (b_5)	λ*imm*434	λ+
λ+	+	+	+	+	+	+	0
λ*immP*22 (3)	0	+	+	0	0	+	+
λ*imm*21*c*I	0	+	+	0	0	+	+
λ*imm*21*c*IIh80	0	+	+	0	0	+	+
λ*imm*21*cIbio*252	0	+	+	0	0	+	+
λ*imm*21 (b_5)p4	0	+	+	0	0	+	+
λ*imm*434	+	+	+	+	+	0	+
Φ80	+	+	+	+	+	+	+
82	+	+	+	+	+	+	+
381	+	+	+	+	+	+	+
424	+	+			does not adsorb		

These experiments represent serial-dilution spot tests + indicate an efficiency of plating of near 1 and 0 represents no plaques seen, to a sensitivity of less than 1 in 10^5 The F′*lac* strains are the same as those used to isolate λ*immP*22, the other lysogens are in strain 594 The λ*imm*21 phages are derived from 21*hyc*I (ref 20) or from λ *b*5 where indicated

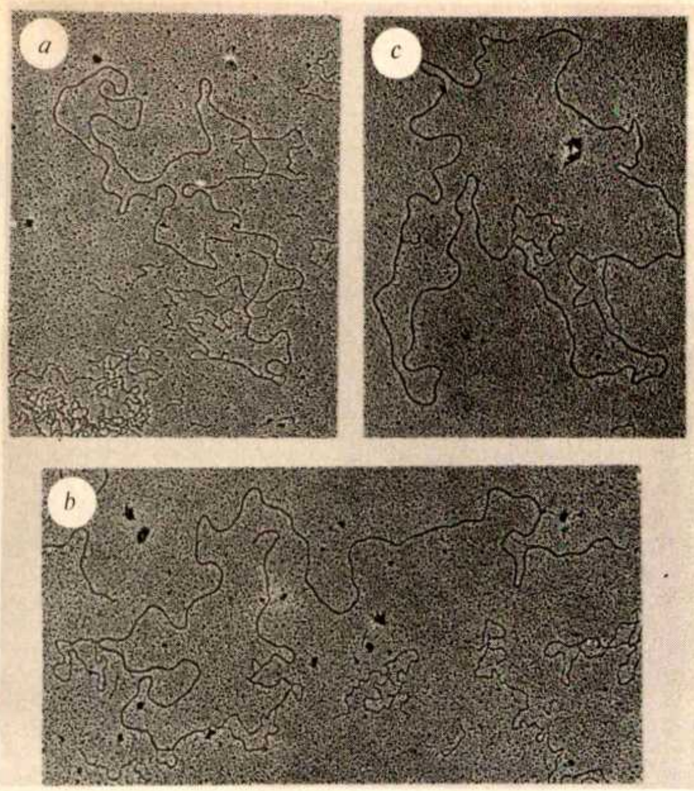


Fig. 2 Electron micrographs of DNA heteroduplexes prepared by the formamide method[34] from purified phage DNA. The b_2 deletion serves as a position and informal calibration marker. *a*, λb_2/λ*imm*P22 heteroduplex molecule; *b*, λ/λ*imm*21b_2 heteroduplex molecule; *c*, λ*imm*21b_2/λ*imm*P22 heteroduplex molecule.

S. Hilliker, unpublished work) indicate that the λ late gene positive regulator (*Q* gene product) can turn on late genes from such prophages. The *Q* genes of λ and P22 may, therefore, have the same specificity.

Physical mapping of λ*imm*P22

The genetic tests presented above indicate that the λ*imm*P22 chromosome is essentially a λ chromosome in which the region from the left of gene *N* to some point beyond gene *P* of λ has been substituted by DNA encoding the analogous functions of phage P22. In addition, λ*imm*P22 and phage 21 have the same immunity specificity, suggesting that 21 and P22 share sequence homology in their immunity regions. To test these views directly, we constructed DNA heteroduplexes between all combinations of wild type λ, λ*imm*21 and λ*imm*P22. Each pair of DNAs differed also by a deletion (*b*2, in the central region of λ DNA) in order to orient the heteroduplex molecules and to provide an internal calibration for measurements. As Figs 2 and 3 show, the heteroduplexes confirm the genetic results; clearly λ*imm*P22 has a DNA substitution in the right arm of λ; λ DNA sequences are replaced by nonhomologous sequences from P22.

The regions of nonhomology between the three pairs of phages (λ/λ*imm*21, λ/λ*imm*P22 and λ*imm*21/λ*imm*P22) all begin at about the same point at the left (14.1—14.6 units from the *b*2 deletion). The *imm*21 and *imm*P22 substitutions therefore have approximately the same lefthand endpoint of nonhomology with respect to λ DNA. Since the *imm*21 substitution replaces the *N* gene of λ, the *imm*P22 substitution must likewise remove the *N* gene of λ. These conclusions confirm the results of recombinational studies shown above (Table 2).

The region of nonhomology between λ and λ*imm*21 is known to end to the left of λ genes *O* and *P* (ref. 26). The righthand endpoint of the major region of nonhomology between λ*imm*P22 and either λ or λ*imm*21 extends well beyond the point of nonhomology between λ and λ*imm*21 (Figs 2 and 3). Comparison with the physical map of Davidson and Szybalski[26] indicates that λ*imm*P22 must have DNA nonhomologous with λ extending to the right of λ gene *P*. Therefore, as suggested by earlier genetic results, λ*imm*P22 has a substitution covering the λ DNA replication genes and origin of replication. We show below that λ*imm*P22 behaves as if it has a *P* gene different from that of λ.

Three small regions of nonhomology between λ*imm*P22 and either λ or λ*imm*21 are found at the far right of the DNA heteroduplexes. The last of these is about 10.0 λ units from the right end of the DNA. If λ*imm*P22 were formed by only two crossovers between λ and P22 DNA, then the crossover on the right must have occurred somewhere to the right of this point. If this be the case, the existence of homology to the left of this point (between the small bubbles) suggests that there is natural homology between P22 and λ DNA in this region. Alternatively, λ*imm*P22 may have been formed by multiple crossovers between λ and P22 DNAs, such that only the three small nonhomologous regions are from P22. We favour the view that λ*imm*P22 resulted from two crossovers, since DNA–DNA hybridisation measurements[4] indicate that λ and P22 DNAs share 18% homology in the right arm.

Whereas the heteroduplex between λ and λ*imm*P22 shows a large region of nonhomology beginning at λ gene *N*, the heteroduplex between λ*imm*P22 and λ*imm*21 shows only a short region of nonhomology. To the right of this region is an equally short region of homology followed by another large region of nonhomology. The existence of a homologous region implies that the *c*I region of λ*imm*21 and the *c*2 region of λ*imm*P22 are not only functionally similar, but are also coded by homologous sequences in the DNA. In support of this placement of the homology to the repressor genes of the two phages, recombinants have been obtained with crossovers between the operators (S. Hilliker, personal communication). The repressor gene sequence homology suggests that the repressors of phages P22 and 21 have a common ancestry.

Control and replication genes

Physical and genetic mapping indicates that the *imm*P22 substitution replaces λ genes *N*, *O* and *P* with analogous genes (24, 18 and 12) of P22 (Fig. 1). This phage can, therefore, be used to study the behaviour of these P22 genes in an *E. coli* host. We have found that λ*imm*P22 hybrids grow normally in certain *E. coli* mutants (*gro*P$^-$, *gro*N$^-$, and *nus*$^-$) which fail to support λ growth (Table 4).

*Gro*P$^-$ mutants of *E. coli* have alterations of a host replication function (*dnaB*) which apparently must interact with the λ *P* gene product for successful λ replication[27]. Since λ*imm*P22 grows in these mutants, the P22 gene product analogous to *P* gene product either interacts with *dnaB* in a different manner or perhaps not at all. It should be noted that *groP*$_{A}$ and *groP*$_{B}$ mutations, known to lie in the

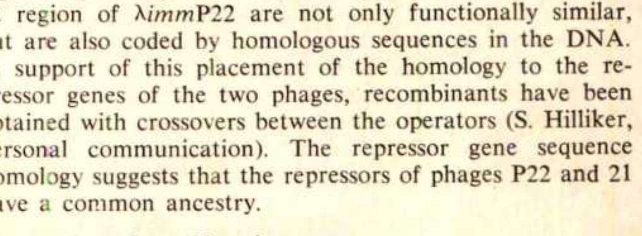


Fig. 3 Summary of measurements of DNA heteroduplexes. All the numbers are averages of at least eight molecules and are normalised to the total length of λ DNA. The locations of the λ genes are taken from Davidson and Szybalski[26].

Table 4 Efficiency of plating on bacterial mutants unable to support growth of λ

Host	λ+	λ*imm*P22	λ*imm*21
gro$^+$	(1)	(1)	(1)
groP$_{A15}$ (*dnaB*)	2×10^{-6}	1.1	5×10^{-6}
groP$_{B560}$ (*dnaB*)	2×10^{-6}	0.7	3×10^{-6}
groP$_{AB756}$	2×10^{-6}	0.07	3×10^{-5}
groN$_{785}$	2×10^{-6}	1.1	0.9
nus$^+$ (42° C)	(1)	(1)	(1)
nus (42° C)	2×10^{-6}	1.0	1.0
nus (30° C)	1.0	0.7	0.7

The *groP* and *groN* mutants are described in refs 27 and 29. *nus* strains are described by Friedman [30]. Figures in parentheses indicate normalisation to this value. All strains are nearly isogenic with either *gro*$^+$ or *nus*$^+$. Two independent λ*imm*P22 hybrids were tested.

dnaB gene[28], do not affect growth of λ*imm*P22, whereas the *groP*$_{AB}$ mutation, which is known to lie outside *dnaB*. (I.H., unpublished result), might have some effect on growth of the hybrid phages.

E. coli mutations *gro*N$^-$ or *nus*$^-$[29,30] block early λ transcription, probably by affecting the function of the early gene positive regulator, N protein. λ*imm*21 is able to grow in these bacteria (Table 4); λ*imm*P22, likewise, grows normally in *gro*N$^-$ and *nus*$^-$ mutants under conditions completely restrictive for λ growth, indicating that the early genes are regulated differently from λ.

From the behaviour in these *E. coli* mutants, it is clear that the early gene control and DNA replication genes of P22 have specificities distinctly different from λs when assayed in the same genetic background.

Implications for virus evolution

The formation of viable hybrids between coliphage λ and the *Salmonella* phage P22 shows that the similarity between these two phages in the arrangement of functions on their genetic maps is the result of a substantial degree of common evolutionary ancestry. We imagine that the hybrid phages were formed by phage-specified or bacterial recombination rather than "illegitimate recombination"[31] because of the relatively high frequency with which they are formed. Not only are viable hybrids formed, but the immunity specificity of P22 and phage 21 are identical, both in function and, to the resolution afforded by heteroduplex mapping, in nucleotide sequence. Similarly, the *Q* gene of λ and the analogous gene (gene 23) of P22 seem to be functionally identical.

A second striking fact about these hybrids is the extent to which nonhomologous genes controlling DNA replication, regulation and immunity of phage P22 remain fully functional when inserted into the chromosome of phage λ. Also notable is the retention of regions of homology flanking nonhomologous segments of DNA which contain nonidentical genes such as those controlling DNA replication. The conclusion which emerges is that evolution of the lambdoid phages (which must now include P22) appears to have proceeded within regions of function which have been selected for the ability to form viable combinations with functional segments from other members of the lambdoid phage group. Since P22 and λ have different bacterial hosts, selection must have maintained this functional and recombinational compatibility for at least the time in evolution since *E. coli* and *S. typhimurium* diverged. It should be noted that *Salmonella* and *E. coli* can exchange genetic material and have similar genetic organisation[35]. Perhaps the enteric bacteria have themselves evolved by a mechanism similar to that suggested for bacteriophages.

Our view of bacteriophage evolution depends on the clustering of related functions on the genetic map[31-34]. The idea that such clustering might be selected through hybrid formation has been advanced as well. But, the idea that the lambdoid phage "races were derived from still other races by re-assortment of unlike segments" the concomitant idea that "presumably both the diversity [of the functional segments] and the capacity to form hybrid recombinants have served a joint evolutionary purpose" was first clearly formulated by Hershey[32]. This idea must now be taken to extend across the species lines of the enteric bacteria.

Heteroduplex studies of the DNA of the lambdoid phages[36,37] and of the lytic T series of coliphages[38,39] support this view of bacteriophage evolution. One can see in the heteroduplexes the whole range of evolutionary divergence: at some points, entire regions of function are nonhomologous and at other points the region of nonhomology may involve only a single gene or even a part of a gene[40]. Although the principle of exchange of functional regions might apply to all bacteriophages, not all phages seem to be in the same group. For example, the temperate coliphages P2 and 186 form viable hybrids with each other (unpublished results of B Egan, H. Younghusband and R. B. Inman) but not, thus far, with the lambdoid phages; similarly, the lytic coliphages T3 and T7 appear to belong to a group separate from that containing phages T2, T4 and T6.

In conclusion, we suggest that evolution proceeds by diversification within segments encoding particular functions: each segment retains functional compatibility and recombinational homology so that reassortment among them is easy and frequent. We imagine that this idea might apply quite generally to bacteriophages, and, indeed, to all viruses, including those of mammals. One might expect therefore that functional hybrids among viruses of quite divergent host species might frequently be encountered.

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LETTERS TO NATURE

Distance to Cygnus X-1

THERE has been much discussion concerning the X-ray object Cygnus X-1 and its optical counterpart, HDE226868, a highly reddened star of type O9 7 Iab (ref 1) The O star is a single-lined spectroscopic binary, and various assignments of the minimum mass of the secondary, taken to be the X-ray object, suggest that the latter is a black hole These conclusions are dependent on the mass of the O star being ~ 20–30 $M_{\odot}$, as would be the case if the star were 'normal' for its luminosity class But the possibility of heating of the primary by the nearby X-ray object, and the spectroscopic indications of mass loss[2], indicate that such an assumption is unsafe In order to determine the mass of HDE226868, a more complete spectroscopic analysis, in which the effective temperature and surface gravity are deduced, and a determination of luminosity (from the distance to the object) are necessary The distance to the object is crucial to the problem, because once the effective temperature and gravity of the O star are known, the mass will vary as the square of the distance An error in the distance by a factor of about two means that the model for the X-ray source need not include a black hole

Accurate estimates of the distance to HDE226868 have recently been claimed by Margon *et al*[3] and Bregman *et al*[4] who give 2 5 kpc Such a large distance favours the black hole model Both works used the reddening produced by interstellar dust as a distance indicator A consistent distance–reddening relationship has been assumed for this region of the sky The evidence for this needs to be examined closely

Interstellar reddening is particularly irregular in the Cygnus region The absorbing clouds of dust collectively form the Cygnus Rift, in which there are many highly reddened OB stars Bregman *et al* showed that HDE226868 is highly reddened compared to the objects—mostly F stars—in their small field of view Apart from the fact that HDE226868 is located extremely near, or actually within, the Great Rift, it is clear that it has photometric properties very similar to reddened O stars in this same region surveyed by Johnson and Morgan[5] This, of course, does not necessarily imply that the object is a normal O star close to the main sequence Plotting the colours of HDE226868 measured by Bregman *et al* ($B-V=+0\ 81$, $U-B=-0\ 30$) on a reproduction of Johnson and Morgan's two-colour diagram (Fig 1), shows that it is not anomalous there are many O stars in this area which are more highly reddened

The relationship between total extinction, A_V (commonly taken to be $3E(B-V)$), and distance for fields in the vicinity of the Milky Way has been investigated by Neckel[6] Neckel was unable to deduce any relationship between A_V and distance within a region centred on $l^{II}=73°$, $b^{II}=0°$, which is extremely close to HDE226868 That survey is, however, considered by Margon *et al* to be inapplicable because Neckel's fields were wide enough to include regions where large changes of absorption could occur over small angular scales Margon *et al* attempted to overcome this difficulty by selecting stars within 50′ of

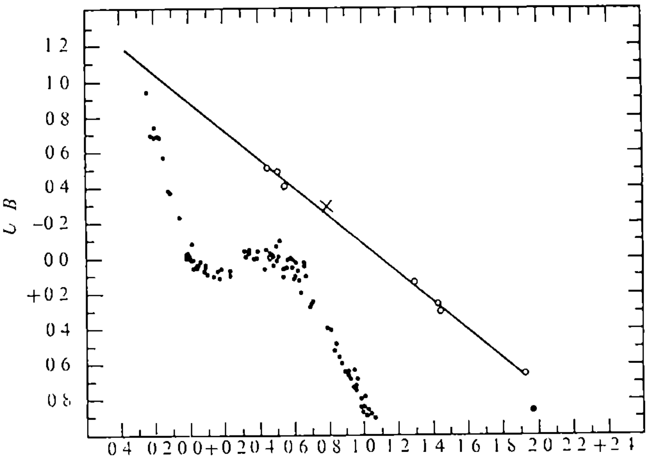

Fig 1 Two-colour plot for reddened O stars in Cygnus from Johnson and Morgan[5] ●, unreddened main sequence stars (from types B1 to K3), ○, O stars in the Cygnus Rift Region, × HDE226868, from the colours of Bregman *et al*[4]

HDE226868 This procedure was clearly not successful, because even at distances below 1 kpc there is an extremely large scatter of points in their plot The extrapolation of such data to greater distances is thus hardly justified The same applies to the plot of $E(B-V)$ against distance for the survey of stars[4] within 30′ of HDE226868 In this case, an independent distance check is provided by the Cepheid V547 Cyg, for which $d=6\ 6$ kpc, about two and a half times greater than would have been estimated from the assumed $E(B-V)$ against d relationship

We therefore conclude that this region is extremely patchy over angular scales as small as 1° This is confirmed by Lynds'[7] survey of dark nebulae which shows not only general patchiness but also the presence of extremely small, dark nebulae The space density of these tiny nebulae may, moreover, be underestimated because of the lack of sufficient background illumination If these objects are representative of the familiar 'Bok globules', then many may have linear dimensions of only 0 05 pc (ref 8), and would therefore subtend only 10″ at a distance of 1 kpc As the reddening produced by the globules is as high as $A_v \sim 5$ magnitudes, large extinction changes can occur over areas of sky which are very much smaller than those covered by either Margon *et al* or Bregman *et al*

Because of the extremely complex nature of the Cygnus region, and the fact that the only independent distance check is so widely in error, the quotation of a distance to ± 16% by Margon *et al* cannot be taken seriously At present we consider that it is over-optimistic to expect reddening measurements to be able to provide the distance of HDE226868 to the required accuracy Until there is a far more accurate determination of the distance of this object it seems premature to suggest that the X-ray source is a black hole

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High energy radiation from white holes

COMPARED to black holes, their time-reversed versions, white holes have attracted little attention from theoreticians Here we point out possible ways in which white holes may be useful to high energy astrophysics

Basically a white hole is an object exploding from a highly dense or a singular state It may be a case of delayed big bang[1] in a Friedman universe, or it may be an instance of a collapsing object reversing implosion to explosion For the latter possibility unconventional equations of state[2] or negative energy fields[3] are required if the theoretical framework is that of general relativity[4] We will take the white hole to be an object exploding from a singularity and obeying Einstein's equations of gravitation subsequent to the singular event Before turning to the astrophysical implications we calculate the spectral features to be expected from a white hole

For simplicity we make the following assumptions (1) The white hole emerges from the singularity as a spherical object of uniform density and zero pressure in the comoving frame of the outward moving particles, (2) the light emitted by the white hole is monochromatic and is being emitted radially outwards from the surface at a uniform rate in the comoving frame of reference, (3) the space-time exterior to the object is empty

The comoving frame of reference in the interior will be denoted by coordinates (r, θ, Φ, t) in terms of which the line element within the object is given by

$$ds^2 = c^2\,dt^2 - S^2(t)\,[dr^2/(1-\alpha r^2) + r^2\,(d\theta^2 + \sin^2\theta\, d\Phi^2)] \qquad (1)$$

where c = speed of light, $S(t)$ is the expansion factor and α a parameter related to the mass M and the comoving radius r_b of the object by

$$2GM = \alpha r_b{}^3\, c^2 \qquad (2)$$

The similarity of equation (1) to the Robertson–Walker line element of cosmology is well known Also, if we change t to $-t$, equation (1) represents a freely collapsing ball of dust

For convenience we will measure t from the instant of explosion so that $S(0) = 0$ For $t>0$, $S(t)$ satisfies the equation

$$S\dot{S}^2 = \alpha c^2\,(1-S) \qquad (3)$$

so that it attains its maximum value $S = 1$ at

$$t = t_0 = \pi/(2c\sqrt{\alpha}) \qquad (4)$$

We will investigate light emission from the white hole in the interval $0<t<t_0$

The space exterior to the white hole is described by the Schwarzschild line element

$$ds^2 = [c^2-(2GM/R)]\,dT^2 - dR^2/[1-(2GM/c^2R)] - R^2\,(d\theta^2+\sin^2\theta\, d\Phi^2) \qquad (5)$$

A typical Schwarzschild observer has R = constant, θ = constant, Φ = constant We wish to calculate the spectrum of radiation from the white hole as seen by a Schwarzschild observer with $R = R_1 \gg 2GM/c^2$. In accordance with our assumption (2) we will take the luminosity spectrum of the white hole as $L\,\delta(\nu-\nu_0)$, where L = constant

To calculate the spectrum received at R_1, define

$$S = \sin^2\theta,\ 0 \leqslant \theta \leqslant \pi/2 \qquad (6)$$

Then θ as a function of comoving time coordinate t is given by

$$t = (2t_0/\pi)\,(\theta - \sin\theta\cos\theta) \qquad (7)$$

The white hole bursts out of the Schwarzschild radius at $t = t_c$, $\theta = \theta_c$, where

$$\sin\theta_c = (\alpha r_b{}^2)^{\frac{1}{2}} \qquad (8)$$

Suppose two successive light signals are sent out from the surface at comoving instants t and $t+dt$ and are received by the observer at R_1 at instants T and $T+dT$ measured in the Schwarzschild coordinate Then a straightforward calculation shows that

$$dT/dt = \sin\theta/\sin(\theta+\theta_c) \qquad (9)$$

So an electromagnetic wave of frequency ν_0 emitted from the surface appears to the receiver to have the frequency

$$\nu = \nu_0 \, [\sin(\theta+\theta_c)/\sin\theta] \qquad (10)$$

A result of this type but in different forms had been obtained earlier by others[5,6] but the above form is suitable for working out the spectrum of the radiation as seen by the Schwarzschild observer Under our assumption (2), $L/h\nu_0$ photons of frequency ν_0 are being emitted per unit t-time from the surface The number emitted in the interval $[t, t+dt]$ is therefore $L dt/h\nu_0$ The same number must be received in the interval $[T, T+dT]$, but with frequencies in the range $(\nu, \nu+d\nu)$ where $d\nu$ is related to dt through equations (7) and (10)
A simple calculation gives

$$dt = (4t_0\nu_0^3 \sin^3\theta_c \, d\nu)/(\pi \, (\nu^2+\nu_0^2-2\nu\nu_0 \cos\theta)^2) \qquad (11)$$

Writing $E = h\nu$, $E_0 = h\nu_0$, the number of photons in the range $[E, E+dE]$ received from the white hole per unit area at $R = R_1$ is given by

$$N(E)dE = (Lt_0/\pi^2R_1^2)E_0^2\sin^3\theta_c dE/(E^2+E_0^2-2EE_0\cos\theta_c)^2 \qquad (12)$$

For $E \gg E_0$

$$N(E) \, dE \cong Lt_0 \, E_0^2 \, (\sin^3\theta_c/\pi^2 \, R_1^2) \, dE/E^4 \qquad (13)$$

The energy spectrum $I(E)$ is given by

$$I(E) = EN(E) \propto E^{-3} \qquad (14)$$

This is the spectrum at the high energy end under the simplifying assumptions made here More general (and perhaps more realistic) assumptions can lead to different types of spectra which we have also worked out We now wish to indicate possible fields in high energy astrophysics where white holes may find applications

(i) The hard electromagnetic radiation from white holes situated at the centres of, say Seyfert galaxies, can be a source of background X and gamma radiation The energy spectrum (14) seems, at first sight to be too steep compared to the observed[7] spectrum $\propto E^{-1\ 2}$ But absorption effects in the gas present in the nuclei surrounding the white hole tend to flatten the spectrum given by equation (14) Detailed calculation with available data[8,9] shows that these absorption effects can in fact flatten the E^{-3} spectrum to $\sim E^{-1}$ form in the range 0 2 keV to 1 keV At lower energies, the ultraviolet radiation seems to be of the right order of magnitude to account for the infrared emission of $\sim 10^{45}$ erg s^{-1} through the dust grain heating mechanism[10]

(ii) The transient nature of X-ray and gamma-ray bursts[11] suggests a white hole origin The shape of the spectrum at the emitting end is likely to be more complicated than the very simple form assumed in the above example In general, however, the spectrum should soften with time

(iii) Although we have worked out the spectrum of photons, it is not difficult to see that similar conclusions will apply to particles of non-zero restmass provided they have very high energy It is possible therefore to think of white holes in the Galaxy on the scale of supernovae, yielding high energy cosmic rays right up to the highest energy observed

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Spectrum of the cosmic background radiation between 3 mm and 800 μm

WE report the successful flight of an experiment designed to measure the spectrum of the cosmic background radiation from $\sim$3 mm to wavelengths shorter than the Planck peak for a temperature of 2 7 K The experiment was launched, to an altitude of 40 km, on March 13, 1974, from Palestine, Texas Because of an on-site computer fault, the complete data were not available to us until May 7, 1974 The instrument has been described previously[1,2] and will be discussed in detail in a future paper, we present here the results of preliminary analysis of the flight data

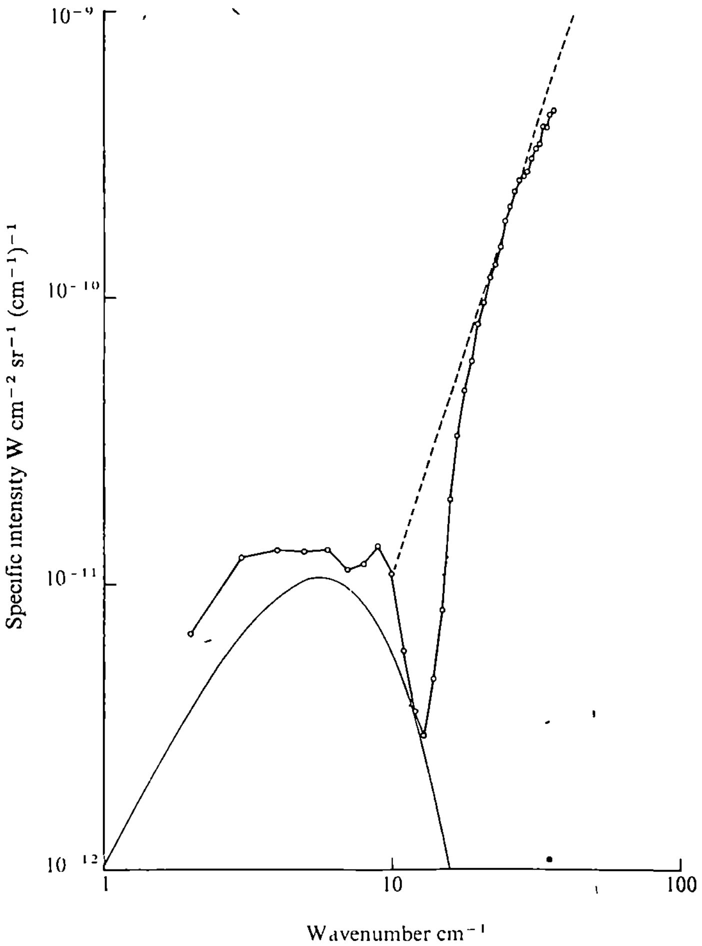

Fig 1 Cosmic background spectrum at 1 cm^{-1} resolution ——o——, Observed spectrum, — — — —, predicted window emission, , window emission using the results of Chantry *et al*[4] Smooth solid line denotes 2 7 K minus 1 4 K curve

The instrument consists of a polarising Michelson interferometer[3] cooled, by a liquid helium bath, to a temperature of 1.4 K. The processed output of the interferometer is the difference between the spectrum of radiation entering the cryostat and the spectrum of the radiation within the cryostat cavity. The interferometer received radiation at a zenith distance of 50° within a cone of semi-angle 3°, the zenith distance of the extremity of the balloon, as seen from the package, was 27°. The interferometer operated in vacuo, separated from the atmosphere by a 50 μm thick window of polyethelene, the measured spectrum should therefore consist of the cosmic background radiation plus emission from the atmosphere and the window. The designed (unapodised) spectral resolution was 0.25 cm^{-1} (Rayleigh criterion). The instrument was calibrated in the laboratory using simulated black body sources at room, liquid nitrogen, liquid hydrogen and liquid helium temperatures, for in flight calibration, we decided to use the emission from the window. The efficiency of the instrument, as a function of frequency, was deduced from the observed room-temperature laboratory spectrum.

One hour of useful time was achieved at float altitude (reached at 0145 CDT), giving 16 interferograms. Total integration time for each sampled point was 19 s. The spectra obtained from these interferograms were averaged at 1 cm^{-1} resolution, the result was divided by the instrumental efficiency function and the high frequency part fitted to the expected window emission between 20 and 30 cm^{-1} to establish a vertical scale. The fitted spectrum is shown in Fig. 1. Also shown is the spectrum of a 2.7 K black body (representing the supposed cosmic background) minus the spectrum of a 1.4 K blackbody (representing the interior of the interferometer cavity).

It is not clear why, below 20 cm^{-1}, the observed spectrum of emission from the window departs radically from that predicted. The prediction of emission, however, is difficult because of the problems of measuring absorption in thin samples. Estimates of absorption vary between experimenters, for example, we show in Fig. 1 the predicted window emission using the absorption figures of Chantry *et al.*[4] We believe, however, that the measured values (E. I. Robson and B. Carli, to be published) used for fitting are correct to (+20, −60)%. It is seen that, if the predicted emission were decreased appreciably, the measured low frequency spectrum would fall below that of 2.7 K minus 1.4 K blackbodies. We have tried the effect of assuming higher temperatures for the interferometer cavity, but to obtain any significant reduction in the height of the predicted background curve requires that the peak emission be displaced to such high frequencies as to exceed the measured values. We are therefore satisfied that the calibration is substantially correct.

Our measured spectrum is a factor less than ~2 higher than that of a 2.7 K background at frequencies below 10 cm^{-1}. A temperature ~$2^{1/3}$ higher than 2.7 K is ruled out, however, by the position of the peak. The excess may well be due to unresolved atmospheric emission[5]. In any case, this first direct spectral measurement shows very clearly the sharp decline in the spectral intensity above 6 cm^{-1}, and it would be very difficult to reconcile our observation with a background temperature higher than ~2.9 K.

We thank our colleagues at Queen Mary College who have contributed to this experiment and the staff of the National Center for Atmospheric Research Balloon Facility for their expert assistance. The project was supported by a grant from the Science Research Council. E. I. R. and J. S. H. acknowledge an SRC Associateship and Studentship respectively.

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A new solar–terrestrial relationship

RECENT suggestions of interrelationships between solar activity, the Earth's magnetic field and the weather[1–3] make more urgent the need to have a reliable method of predicting sunspot activity.

Most attempts to develop prediction techniques have been associated with empirical relationships derived from the preceding behaviour of many sunspot cycles, but none of these has been really successful over any extended period. Wood[4] has re-opened consideration of the possibility[5] that solar activity may be controlled by the tidal influence of the planets. In the course of a detailed study[6] of 25 yr of geomagnetic data it was found that the phase of the solar diurnal variation of the horizontal field on quiet days, Sq(H), exhibits a marked solar control: the variability of the phase is most marked in winter and at sunspot minimum epochs. The solar cycle behaviour has now been studied over an 89-yr period from 1884, the earliest year for which International Quiet Days have been designated, and a consistent tendency has been found for the magnetic data at sunspot minimum to anticipate the magnitude of the subsequent sunspot maximum.

Both the amplitude and phase of the Sq variation vary considerably from day to day, even on very quiet days. The variability in the amplitude has been well studied, but relatively little work has been published on the variability in the phase. Here, the phase variability is discussed. Figure 1 shows the diurnal variation of the occurrence of the time of daily minimum horizontal field for all the international quiet days in the period 1884–1972. Although the most common time of H-min is between 1030 and 1130 LT, there is a wide range of appropriate times. Days for which H-min occurred within 2½ h of 1100 LT

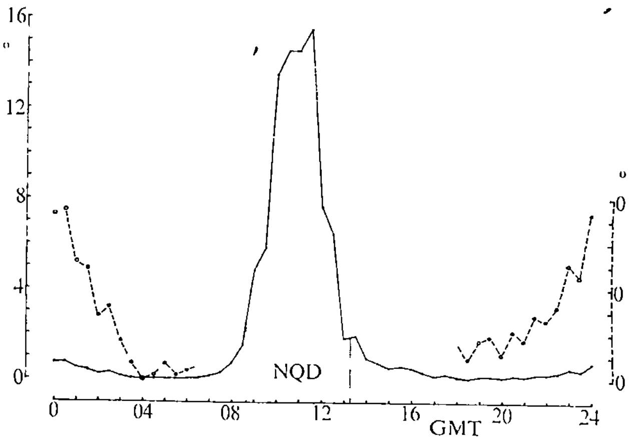

Fig. 1 Diurnal variation of the percentage number of international quiet days of the period 1884–72 for which the daily minimum horizontal magnetic field occurred within each half-hour of the day at Greenwich/Abinger/Hartland. Dashed curve is for night-time data plotted on a ×10 ordinate scale, indicated on the right-hand side.

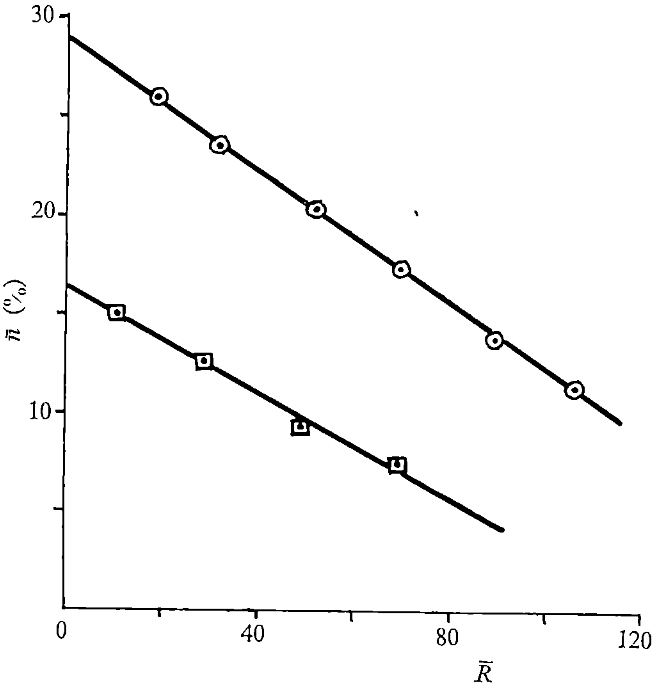

Fig 2 Average percentage occurrence of AQDs ($\bar{n}$) as a function of average sunspot number ($\bar{R}$) derived from annual mean 3-yr smoothed data averaged over increments of 20 in R Data are the same as in Fig 1, subdivided into two periods moderate activity, 1885–1936 (squares), strong activity, 1937–71 (circles)

are styled 'normal quiet days' (see ref 6) These are included within the shaded region marked NQD in Fig 1 All days outside this region are defined as 'abnormal quiet days' (AQD)

The dashed curve in Fig 1 represents a replot of the points for the night-time period on a × 10 ordinate scale, it seems that within the AQD category there is a subsidiary maximum occurrence of near-midnight minima in H At such times the conductivity of the E region of the ionosphere is so low that essentially no electric current flow can occur, so that the reduction in the horizontal magnetic field cannot be ascribed merely to positional changes of the atmospheric Sq current loops Evidence is presented elsewhere[7] for a connection between the occurrence of the more moderate AQDs and changes in the structure of the terrestrial atmosphere and the orientation of the Sq current loops, but it seems likely that the more extreme types of AQD have an extraterrestrial (probably solar) cause

It was previously found[6] that the occurrence of AQDs is primarily a winter phenomenon, and examination of the detailed seasonal variation over the period 1884–1972 shows that the most extreme AQDs occur exclusively in winter Confirmation of this inverse relationship to solar radiation is provided by the negative correlation between AQD occurrence and sunspot number, illustrated in Fig 2 In this diagram the data have been smoothed over 3 yr and the mean AQD count determined for each successive interval of 20 in sunspot number

For each group of data in Fig 2 (1885–1936 and 1937–71) there is a remarkably linear increase in the occurrence frequency of AQDs with decrease in solar activity, such that the occurrence is primarily confined to times near solar minimum Extrapolating the Fig 2 data shows that the mean occurrence frequency of AQDs for 'zero sunspots' for the 1885–1936 cycles is 16 5%, and for the 1937–71 cycles it is 29% These differences probably emphasise the relative crudity of the sunspot number as an index of solar activity The earlier group is for a period of relatively low sunspot maxima and low minima, the later group has high sunspot maxima and relatively high sunspot minima, and it seems likely that the bodily displacement of the lines in Fig 2 is conditioned by varying thresholds of solar activity which are not apparent in the values of sunspot numbers themselves The 'AQD count' parameter is interesting since it is an index which maximises at sunspot minimum, and it is conceivable that it thereby provides a rather sensitive measurement of the depth' of solar minimum not evident in the conventional sunspot number

Further insight into the solar cycle control of AQD occurrence is provided by an examination of the long-term behaviour over the eight separate solar cycles investigated Figure 3 shows a 3-yr running mean of the average quarterly values of AQD count (solid curve) and sunspot number (dotted curve) The inverse correlation between the two curves corroborates the result of Fig 2, a new feature is the striking variation in the amplitude of the maxima of the AQD count (at sunspot minima) such that the change from one cycle to the next is always in the same sense as the change in amplitude of the subsequent sunspot maxima This effect is better illustrated in Fig 4 which shows 3-yr running mean curves of the annual values of both parameters, plotted so that the time scale for sunspot number is 6 yr ahead of that for the AQD count The effect is consistently observed over the ups and downs of the past eight solar cycle maxima, and is particularly evident over the period 1920–72 when after three consecutive increases in the size of the AQD count and the subsequent solar maxima (culminating in the record IGY maximum) the following maximum was significantly smaller for both parameters

It is now possible to rationalise the two lines obtained in Fig 2 when concurrent values of AQD count and sunspot number are plotted against each other Figure 5 shows a plot of the amplitudes of the cycles in each parameter, read from Fig 4, using both the ascending and descending parts of each cycle, where each AQD count amplitude is associated with the corresponding sunspot number amplitude one half-cycle ahead The approximately linear relationship shows that each change in AQD count is approximately mirrored by a proportional change in sunspot number during the corresponding part of the subsequent cycle

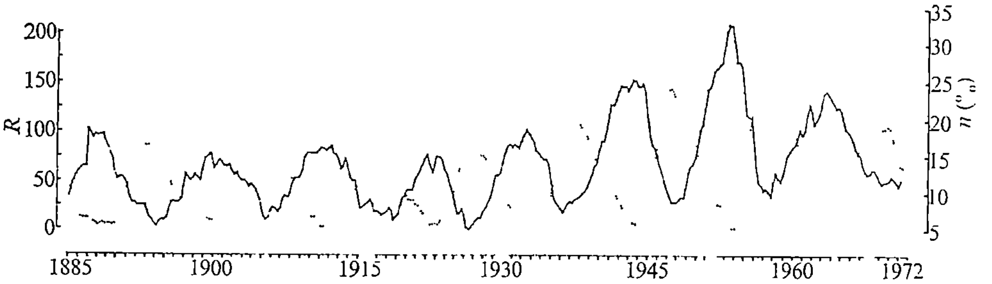

Fig 3 Three-yearly running mean of the quarterly occurrence of AQDs (n, solid curve) and of sunspot number (R, dotted curve) for the data as in Fig 1

If this relationship proves valid it implies that the Sun 'breathes' with an 11-yr period, such that the size of a solar activity maximum is determined at the very beginning of a cycle, or perhaps the very end of the preceding cycle, from the 'depth' of the solar minimum This quantity is essentially unmeasurable by the conventional sunspot number, and may be insensitive of detection by other indices of solar activity which decrease at this time The AQD count index, based on a simple analysis of the variation of the Earth's magnetic field on quiet days in winter, seems to provide a highly sensitive index of the extent of solar minima Detailed examination of the various cycles in

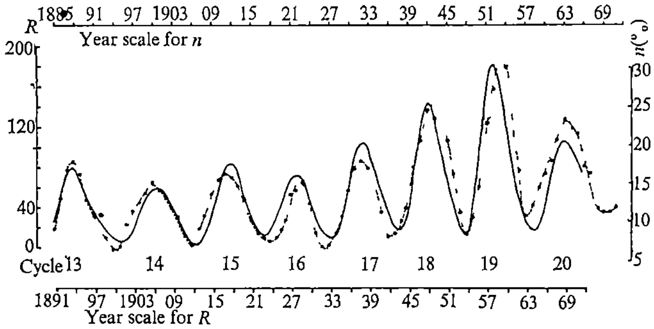

Fig. 4 Three-yearly running mean of the annual occurrence of AQDs (*n*, dashed curve, upper abscissa scale) and of sunspot number (*R*, solid curve, lower abscissa scale) for the data as in Fig 1 Note the 6-yr displacement of the time scales

Fig 3 reinforces the conclusion that an apparently close relationship exists between the two parameters separated by an interval of 5–6 yr For example, there is a marked tendency for some maxima in AQD count to be double-humped, with peak separations of about 2 yr Double maxima are a feature[8] of the solar cycle (although not always evident in sunspot number) and have been interpreted as the result of the superposition of two processes having different physical properties It is tempting to suggest that the extra large and single-humped sunspot maximum of 1957–58 arose from a coincidence of the two peaks, and it is noteworthy that the preceding AQD count maximum in 1953–54 also exhibits a single sharp peak

There are obvious difficulties in accounting for a time constant as long as 5–6 yr from the solar side But it is now evident that the solar cycle is a much more complicated periodicity than that indicated by the variation of any one index of activity The cycles in solar wind intensity and velocity, coronal green line intensity and coronal shape, and sunspot number all differ in phase from one another, with a maximum difference of at least 3 yr (ref 9) One model[10] of the Sun's magnetic field proposes that the magnetic polarity of solar polar regions changes sometime during the decline from sunspot maximum to sunspot minimum, and a solar activity centre is considered to have a 'history' related to solar happenings during the preceding years The interplanetary field near the Earth is largely controlled by the solar polar field and, in keeping with the above result, the phase of the annual variation of the interplanetary field changes about 2 7 yr after sunspot maximum[11]

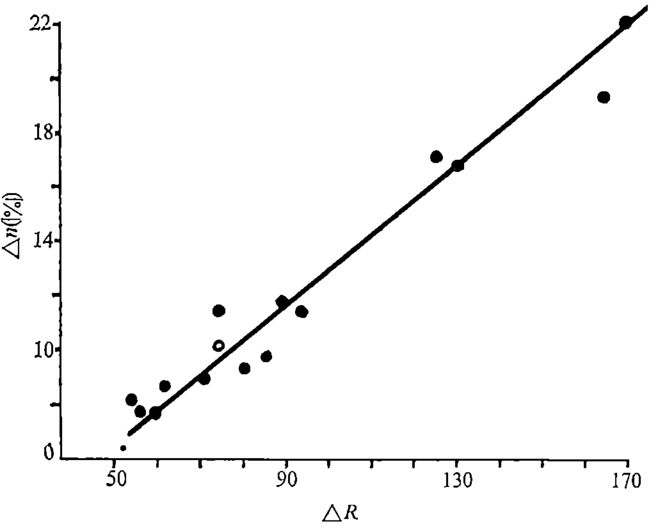

Fig 5 Amplitudes of the cycles in AQD occurrence (Δn) and sunspot number (ΔR) one half-cycle ahead, read from Fig 4 (Open circle is for the ascending part of cycle 13 and involves an estimate of the value of Δn, since no magnetic data are available for the time of the AQD minimum)

From the terrestrial magnetic side it is difficult to speculate on possible mechanisms while the essential cause of the AQD phenomenon remains obscure Comparison of Sq data for observatories spaced 1,000 km apart has shown[6] that 80% of the AQDs are common at the two sites in winter and at sunspot minimum, the agreement falls to 35% in summer and sunspot maximum This suggests that when the most variability is encountered it influences larger areas of the Earth Thus, although the AQD index, as presented, is derived from data for one station only, it reflects a regional or planetary phenomenon The unusual minima in the horizontal magnetic field at a station tend to occur around 0000 UT and are generally additional to the normal minima around 1100 LT which are associated with the ionospheric Sq current system[12] So it seems very likely that the cause of the AQD incidence is extraterrestrial, and is probably solar in origin A likely line of communication between the Sun and Earth seems to exist in the interplanetary field The varying 'sector structure' of this field, which is related to the solar magnetic field, can be seen clearly in the behaviour of the geomagnetic field at high latitudes[13] It has also been proposed[14] that during quiet days the magnitude of the total interplanetary magnetic field is partly responsible for the day-to-day variability of Sq (range rather than phase) at low latitudes Furthermore, the sector structure of the interplanetary field shows a solar cycle variation[15], and exhibits a particularly stable structure at sunspot minimum[16]

There remains the possibility that the relationship reported here is fortuitous, albeit being obeyed for a tantalisingly long period The ultimate test must await much more extensive data, or the realisation of a sound scientific basis for such a connection along the lines suggested above Meanwhile, the index could probably be refined considerably by basing it on magnetic data from several geographically separated stations, and by defining the incidence of an AQD more precisely It seems (Fig 4) that there is a long term secular trend in AQD occurrence, evidenced by the almost continual increase in the size of the minimum count of each cycle over the period covered

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Rainfall, drought and the solar cycle

THE present drought devastating the Sahel, India and Ethiopia has been ascribed to long term climatic changes Winstanley[1] and Lamb[2] suggest that the general circulation of the atmosphere is slowly changing, resulting in an equatorward shift of the principal climatic belts If these shifts are part of a 200-yr cycle, as Lamb suggests, severe droughts should become more common during the next 60 yr But at Addis Ababa the 11-yr solar cycle seems to have a greater influence on rainfall variations and so the timing of droughts, than does the long term cycle

In Fig 1 we compare the variation of annual rainfall recorded at Addis Ababa during the past 72 yr (refs 3–5 and unpublished data from Addis Ababa Geographical Observatory) with the Zurich sunspot number Both sets of data have been smoothed by plotting 3-yr running means The rainfall clearly follows a cyclic pattern in which rainfall peaks and troughs precede the sunspot peaks and troughs by a few years This agreement is not caused by the smoothing technique used comparing each year's unsmoothed rainfall and sunspot values the correlation coefficient, *r*, is 0 21 (significant near the 5% level) If each year's sunspot value is compared with the previous year's rainfall, $r=0\ 29$ (significant near the 1% level) and if compared with the rainfall 2 yr before, $r=0\ 33$ (significant at better than the 1% level) Thus, there is a strong statistical correlation between Addis Ababa rainfall and sunspot number, the rainfall peaks leading the sunspot peaks by an average of 1 3 yr, the actual values for the 7 cycles being 1, 2, 3, 2, 1, 1, 0 yr It is noteworthy that, in a reported correlation between atmospheric ozone content and sunspot values[6], the ozone troughs preceded the sunspot peaks by 1 5 to 2 yr

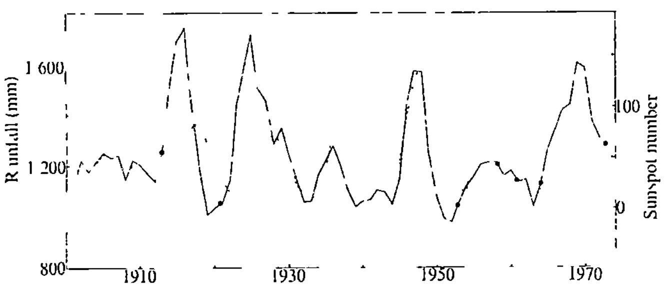

Fig. 1 Three-year running means of Addis Ababa rainfall (———) and three-year running means of Zurich sunspot numbers (— — —) ●, Known Ethiopian drought years In cases where discrepancies occur between rainfall data from refs 3 and 5 preference has been given to ref 3

The phase relationship of the two curves of Fig 1 is well maintained, but the amplitude relationship is more complicated There is a tendency for the magnitude of the rainfall peak to be inversely related to the magnitude of the sunspot peak (Fig 2*a*) A similar relationship appears for the trough values (Fig 2*b*) But since two of the data points on each graph disagree with this trend there is not sufficient evidence to state this relationship definitely

If this rainfall/sunspot cycle relationship continues then, using King-Hele's[7] prediction of 1978 as the peak year of the next solar cycle, we would expect Addis Ababa rainfall to increase until about 1976–77 and then decline to a minimum about 5 yr later Further, if the relationship shown by Fig 2*a* holds, then the predicted value of 110 for the next sunspot number peak[7] gives a value of about 1,600 mm for the next rainfall peak

It is important to find whether such a relationship applies for Ethiopia as a whole, and for other drought afflicted areas of Africa Instrumental rainfall records have been obtained only recently in most areas of Ethiopia, but a 70-yr record exists for Asmara A preliminary study of this shows little correlation with solar cycle before 1930, but a negative correlation after that date

To support the scanty instrumental data on rainfall, an indication of past rainfall troughs can be obtained from historical records of drought, and drought-induced famines To be recorded by travellers or historians a drought would have to have been severe enough, and lengthy enough, to affect seriously the life of the country, and not have been merely an isolated year of poor rainfall Figure 3 is a histogram of presently known Ethiopian drought years, since 1540 AD, as a function of years from sunspot minimum Droughts have occurred in each of the years of the sunspot cycle, but 14 of the 27 drought years occurred in the 3 yr centred on sunspot minimum Six more occurred in the years following minimum, possibly representing droughts caused by an accumulation of bad years (the effects of a long deficiency of rain persist during the first years of improved rainfall) Thus, there is some evidence that the rainfall/solar cycle relationships observed at Addis Ababa has applied for the past few hundred years over highland Ethiopia as a whole

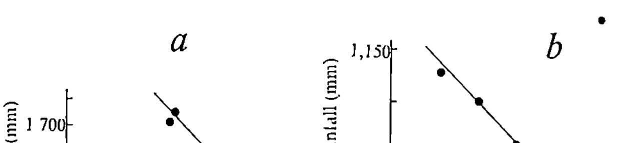

Fig 2 *a*, Rainfall cycle peak values against corresponding sunspot cycle peak values The line is a least squares fit to the solid points only *b*, As for *a* but for corresponding trough values

It may be significant that the early 1930s coincided with several changes in climate/solar cycle correlations From this time the Asmara rainfall shows some solar correlation, and for Addis Ababa, the time lag between rainfall and sunspot peaks decreased There was, at the same time, a phase change in the relationship between sunspot values and 8 m depth earth temperatures in the UK, and also phase changes in equatorial pressures and temperatures[8] Additionally, the level of Lake Victoria showed high solar cycle correlation from 1890 to 1930, but the relationship broke down after that time[8] These changes may be related to the proposed 200 and 700-yr climatic cycles which peaked at that time[1]

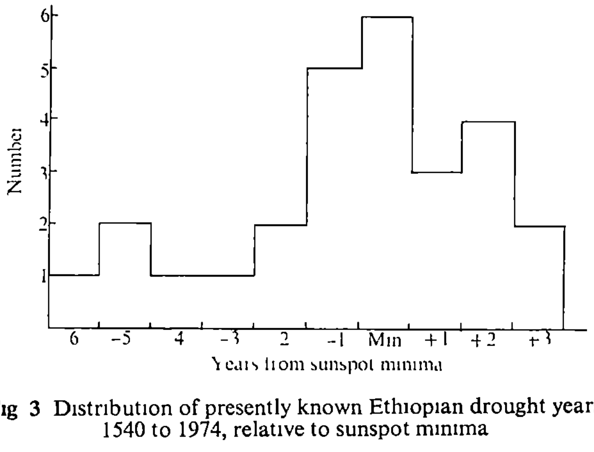

Fig 3 Distribution of presently known Ethiopian drought years, 1540 to 1974, relative to sunspot minima

An intensive study of the Indian monsoon rains[9], based upon the 50-yr records of many stations, has found significant correlations with solar cycles In regions of orographic rainfall near the Himalayas, strong, positive correlations are found, whereas in some other regions there are negative correlations Zones of positive and negative correlation are separated by areas of little correlation The Indian data illustrate that the solar influence on rainfall is spatially modulated, in unknown ways, resulting in systematic variations of the sign and degree of correlation Similar solar cycle influences should be looked for in the rainfall records of the Sahel and elsewhere

If this sunspot–rainfall relationship is substantiated by data from other stations in the Sahel and India, governments and relief organisations will have a valuable warning of periods of greatest drought danger

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Dynamic implications of mantle hotspots

MOVEMENT of lithosphere plates over hotspots which are fixed relative to the mantle could explain the origin of island chains and aseismic ridges[1-4] Observations of phase velocities against epicentral distances for Tonga–Samoa earthquakes[5] show anomalies which can be interpreted as a radial and lateral inhomogeneity in the lower mantle beneath Hawaii That indicates the possible existence of deep mantle plumes

Here, I consider some implications of the mantle plume hypothesis on the basis of a simplified flow model I represent the flow of the plume by viscous flow through a pipe The Navier–Stokes equations, assuming steady state, fluid incompressibility and uniform viscosity, reduce to a simple form for the case of pipe flow The maximum flow velocity, V_z, given by the exact solution of these equations, is

$$V_z = (dp/dz)\,(a^2/4\eta) \qquad (1)$$

where a is the radius of the plume, the flow is along z axis and η is the coefficient of viscosity

$$\text{The volume flux} = (\pi a^2/2)\ V_z \qquad (2)$$

The associated energy equation, based on the same assumptions as for Navier–Stoke equations, also yields an exact solution for pipe flow, giving the temperature δT, generated by the flow

$$\delta T = \eta\ V_z^2/4K \qquad (3)$$

where K is the coefficient of thermal conductivity

Table 1 lists the temperature differences and the volume flux for a range of velocities of plume flow

The values used in computing Table 1 are $\eta = 3 \times 10^{21}$ g cm^{-1} s^{-1}, $K = 10^{-2}$ calorie °C^{-1} cm^{-1} s^{-1}, and the plume radius, a, = 75 km The most frequently quoted[2] vertical velocities of plume flow are 2 myr^{-1} It is clear that such velocities will generate unacceptably high temperatures if the frictional viscosity is of the same order of magnitude as present estimates of the mantle viscosity

With a speed of flow of 2 m yr^{-1} each plume would bring up about 18 km^3 yr^{-1} of mantle material Twenty plumes will bring up a total of 360 km^3 yr^{-1} That would be the total flux of the material moving away from the rise crests Lithosphere is created at a rate of about 170 km^3 yr^{-1}, which is the flux of counterflow towards the ridge crests The resultant drag exerted by the flow on the lithosphere will be in the direction of plate motion, that is, away from the rise crests[2] The drag can be increased by increasing either the number of plumes, or their vertical flow speeds or dimensions

If, however, the flow speed is reduced to, for instance, 3 cm yr^{-1}, temperatures would become reasonable But the total flux from 20 plumes would be reduced to about 6 km^3 yr^{-1} Then, because the total flux of flow towards the rise axes would be many times the flux away from them, the net stress on the plates would tend to close the plates rather than move them apart Thus, as well as the necessity for a source to provide adequate motive forces for plate motions it becomes necessary to find additional stresses to overcome the closing trend That adds unnecessary complexity to the problem

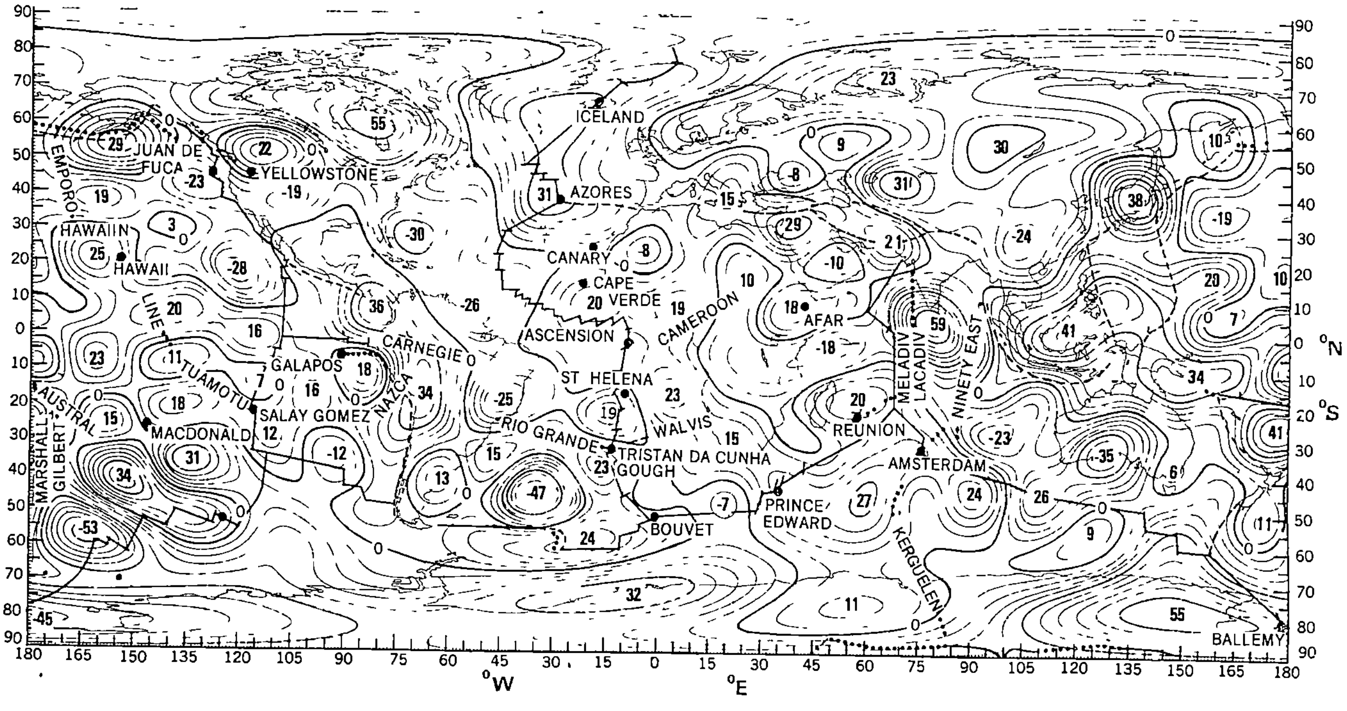

Fig 1 Relationship between proposed hotspot localities and gravity anomalies (mGal)

Table 1 Typical temperature differences and volume flux for a range of flow velocities

Flow velocity (cm yr^{-1})	Temperature difference (°C)	Volume flux per plume (km^3 yr^{-1})
200	6 75 × 10^4	18
100	1 69 × 10^4	9
10	1 69 × 10^2	9 × 10^{-1}
3	≈ 15	2 7 × 10^{-1}
1	≈ 2	9 × 10^{-2}

Therefore, either an alternative way of keeping down temperatures must be found or the idea that mantle plumes have any significant role in plate tectonics must be rejected

It may be possible to introduce variations in viscosity and conductivity, although I have assumed uniform viscosity and conductivity Both, however, are temperature dependent Therefore, the temperature, δT, generated by the flow, will be affected, but it is uncertain whether the effect would be sufficient to reduce the temperatures to a reasonable level

Thus, assuming that the results of Table 1 are correct to an order of magnitude then, if the temperatures are to be kept reasonable, some other parameter in equation (3) must be adjusted The only reasonable possibility at this stage is a reduction in the velocity of the vertical flow of the plume, perhaps accompanied by some increase in its diameter Unfortunately, that makes the mantle plume hypothesis much less attractive

It has been contended[2] that the viscous drag exerted on the bottom of the lithosphere by a typical plume, will result in the doming of lithosphere, producing a regional topographic high and a gravity excess over the area Most hotspots do indeed lie over areas of positive gravity, though very few lie directly over gravity maxima, Fig 1 shows the probable locations of the hotspots, superimposed on isostatic gravity anomalies based on gravity Model GEM 4 (Khan, M A, and Lerch, F, *et al*, unpublished) A significant number of hotspots, however, show association with negative gravity, Galapagos, Juan de Fuca, St Helena, Macdonald and Salay Gomez That contradicts the general claim that plume locations are always associated with positive gravity

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Λ-type doubling in the CH molecule

Almost a year ago we set out to calculate an accurate value of the Λ-doubling in the lowest rotational level of the $X^2\Pi_{\frac{1}{2}}$ state of CH, to assist the radioastronomers, who had been searching for this species in interstellar space for some years The value we have obtained, which is reported here, represents a substantial improvement on the results of terrestrial experiments[1,2], but its significance is reduced by the astronomical observation of the species, which was reported during the course of this work[3] The result of the latter experiment indicates just how close our calculation comes to predicting the actual splitting and clearly underlines the power of the methods now available in theoretical chemistry

Van Vleck[4] has shown that the Λ-doubling of the rotational levels of a $^2\Pi$ state may be represented as a perturbation by $^2\Sigma$ states To second order, the splitting may be expressed in terms of two parameters, $(\frac{1}{2}p+q)$ and q For the lowest level of a regular $^2\Pi$ state, for which $J = \Omega = \frac{1}{2}$, the doubling is simply $2(\frac{1}{2}p+q)$, where

$$p = 4\sum\frac{\langle{}^2\Pi\,|H_{\mathrm{so}}|\,{}^2\Sigma\rangle\langle{}^2\Pi\,|B(L^+ + L^-)|\,{}^2\Sigma\rangle}{E_\Pi - E_\Sigma}$$

$$\text{and } q = 2\sum\frac{\langle{}^2\Pi\,|B(L^+ + L^-)|\,{}^2\Sigma\rangle^2}{E_\Pi - E_\Sigma}$$

where the summation extends over all vibrational levels of all $^2\Sigma$ states, the contribution from $^2\Sigma^-$ being taken as negative Matrix elements are taken over both electronic and vibrational wave functions H_{so} is the spin-orbit coupling operator, and B is the operator $h/8\pi^2\mu r^2$

An *ab initio* calculation of the parameters p and q was performed for CH by Hinkley *et al*[5] It was assumed that the electronic matrix elements were independent of internuclear distance, so that vibronic matrix elements could be factorised into electronic and vibrational integrals The Hartree-Fock orbitals calculated for the $X^2\Pi$ state by Cade and Huo[6] were used, the invarient orbital approximation was made, so that Slater's rules might be applied to the matrix elements Vibrational wavefunctions were calculated numerically from Morse curves Only the contributions of the $B^2\Sigma^-$ and $C^2\Sigma^+$ states were considered The results are shown in Table 1, they are some 20% too low

In the present work, the calculation of Hinkley *et al* was modified in several ways Herzberg and Johns[7] have shown experimentally that both $^2\Sigma$ states have maxima in their potential curves, so that the latter hardly resemble Morse curves Vibrational wave functions were therefore calculated from the extended configuration interaction potential curves of Lie *et al*[8], which represent the states rather well The functions were calculated for the hypothetical case where $J = 0$, and the effects of predissociation on excited vibrational levels were ignored The vibrational integrals obtained from these wave functions were combined with the electronic matrix elements of Hinkley *et al* to produce the result labelled (a) in Table 1

Table 1 Λ-type doubling in the $X^2\Pi_{\frac{1}{2}}$ ($v = 0$, $J = \frac{1}{2}$) state of CH

		$\frac{1}{2}p+q$ (cm^{-1})	q (cm^{-1})	Doubling (MHz)
Hinkley *et al*[5]		0 0447	0 0254	2,680
This work	(a)	0 0430	0 0245	2,576
	(b)	0 0447	0 0274	2,680
	(c)	0 0563	0 0386	3,374
	(d)	0 0552	0 0379	3,311
Terrestrial experiment[1 2]		0 0563	0 038	3,374 ± 20
Astronomical experiment[3]		0 0556	—	3,335 47 ± 0 01

The error introduced by the invariant orbital approximation was then investigated A separate set of SCF orbitals was constructed for each of the $^2\Sigma$ states from the basis set of Cade and Huo, using the ALCHEMY program[9] Electronic matrix elements were then calculated using a program written by J A Hall[10] The values now obtained for the Λ-doubling parameters (b) show that further improvements were necessary The electronic wave functions were further modified by configuration interaction, six configurations were included for the $X^2\Pi$ and $B^2\Sigma^-$ states, while eight were used for the $C^2\Sigma^+$ state Electronic matrix elements were again calculated by the Hall method, the result (c) was in close agreement with the experiment

Finally, the C I wave functions were calculated over a range of internuclear distances The electronic matrix elements were then integrated numerically over the vibrational wave functions The results (d) are within 1% of the experimental values

The Λ-doubling is predicted more accurately by this calculation than by terrestrial experimental estimates

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Drag-reducing polymers and liquid-column oscillations

We report here on one of many anomalies which occur when drag-reducing polymers are dissolved in water[1–4] the damping of manometer-type oscillations

Our apparatus was made from clear PVC tubing (nominal bore 1 3 cm) mounted vertically on a wooden board The tube was partially filled with liquid, thus creating a rudimentary manometer The basic experiment consisted of giving the liquid column an initial displacement (25 cm in all cases) from equilibrium and then counting the subsequent oscillations Graphs of decaying amplitude of oscillation against time were obtained by filming the process at a speed of 18 frames per second Two pipe configurations were studied a semicircle, of radius 29 cm, and a conventional U-tube with the bend having a radius of curvature of 4 5 cm The length of the liquid column (88 cm) was the same in both cases

In each case the experiment was performed first with water and then with increasing concentrations of drag-reducing polymer in aqueous solution The additives used were polyethylene oxide (Polyox WSR 301 and WSR N3000) and polyacrylamide (Separan AP 273 and MG 200)

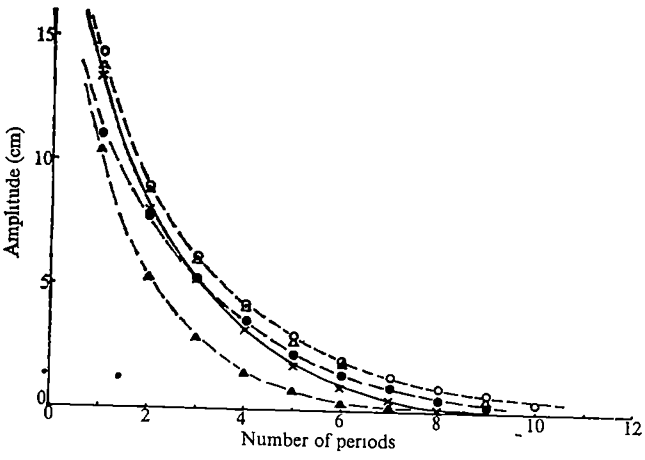

Fig. 1 Amplitude of oscillation against time for Polyox WSR 301 in a semicircular tube ×, Water, ○, 10 p p m WSR 301, △, 25 p p m WSR 301, ●, 250 p p m WSR 301, ▲, 500 p p m WSR 301 Each period=1 6 s

We found that both grades of polyethylene oxide reduced the damping (relative to water) at quite small concentrations, whereas the polyacrylamides, although effective drag-reducers in turbulent flow, gave no measurable reduction

For small additive concentrations (Polyox WSR 301), a marked reduction in damping (relative to water alone) was evident after the first few oscillations (Fig 1)

The behaviour at larger concentrations was particularly interesting For the first few oscillations the damping actually increased (presumably reflecting the increase in solution viscosity) but for the smaller amplitude motions a reduction in damping again occurred (Fig 1) These results suggest that, during the first few oscillations, the flow is controlled by the viscosity of the solution alone

The corresponding results for the U-tube were very similar, there is little apparent difference between the two flow configurations (Fig 1)

Counting the total number of oscillations does, however, reveal that the additives have surprising quantitative effects on the damping If the total number of oscillations with water is N_w and with polymer solution N_p, the percentage increase in the number of oscillations can be defined as

$$100\ (N_p - N_w)/N_w$$

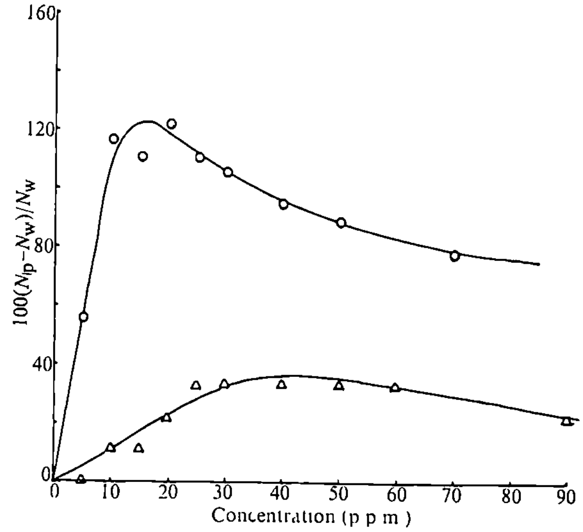

Fig. 2 Variation of damping reduction (assessed by observing the percentage increase in the total number of oscillations) with concentration of Polyox WSR 301 ○, Semicircular tube, △, U-tube

Results for the semicircular tube (Fig 2) show that the total number of oscillations is more than doubled in the concentration range 10–30 p p m On the other hand, the U-tube (Fig 2) shows a much smaller peak enhancement of about 30–40%, over the broader range of 25–65 p p m

Polyethylene oxide tends to adhere to the pipe wall In an attempt to assess the importance of this wall coating, a tube which had previously been used with solution concentrations of up to 500 p p m was rinsed out, to remove any remaining drops of polymer solution and the tests were then repeated, beginning with water It was found that, when water was added to a coated tube, there was a considerable degree of enhancement (relative to water in a clean tube) To exclude the unlikely possibility that a portion of the polymer coating had dissolved in the fresh water, the sample was decanted after a marked reduction had been observed. It was then placed in a clean PVC tube and the experiment was repeated and no significant reduction in damping was observed. It is, therefore, difficult to avoid the conclusion that observed reductions in damping occur because of an adsorbed layer of polymer additive on the tube wall, rather than because of the viscoelastic properties of the actual solution.

Our results indicate that very small amounts of polyethylene oxide can produce quite large reductions in the degree of damping of manometer-type oscillations. The failure of polyacrylamide to show any enhancement may result from the fact that it does not seem to coat the tube, or it may conceivably be a matter of time scales. Unfortunately, as the period of oscillation is proportional to the square root of the length of liquid in the tube, an investigation of much smaller time scales would have its difficulties.

The much greater reduction in the degree of damping found with the semicircular tube is presumably because the tube is entirely curved, whereas the U-tube is only partially so. This would be consistent with previous results[5] for steady flow in curved pipes, in which polymer additives resulted in marked reductions in drag.

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1/*f* noise with a low frequency white noise limit

One of us (A.A.V.), who had recently been studying channel models for electrical fluctuations caused by potassium transport through nerve membrane, noticed a possible analogy with the flow pattern of grain through an hourglass of unusual shape designed by F. Vaudan, Paris. The hourglass was filled with small grains (0.1 to 1.0 mm diameter) of coloured glass and differed from ordinary hourglasses in the length of the pore connecting the two compartments which was drawn out to about 25 cm; about 80 times the smallest inner diameter (3 mm). When the hourglass was turned, the grain did not flow steadily but exhibited slow irregular density fluctuations (Fig. 1). With a view to the analogy with potassium channels in excitable membranes, we decided to examine comparative power spectra[1-3].

In a series of experiments to measure spectral properties of the grain flow, a laser beam (Spectra-Physics model 155) was sent through the stem of the hourlgass and then through a small hole (0.2 mm diameter) placed in front of and close to a photosensitive cell and a dc amplifier.

Light-beam switching resulted in square-waves with a resolution time of about 10^{-4} seconds for the system of cell and amplifier. Another test using a white-noise modulated light source with a white band between 1 and 5,000 Hz showed that the system response was flat within the available range.

In the resulting pulse sequence, most of the pulses had comparable shapes (about triangular, or triangular with clipped peaks when the grains completely blocked the beam during part of their passage) and durations which varied from about 10^{-3} s to 10^{-2} s. After amplification, the power spectrum was calculated (Fig. 2, curve A). Its shape did not change for measurements through different levels of the stem (higher up, where the stream is dense and the light shines through occasionally, or lower down, where individual particles pass through the beam).

The mechanism responsible for the spectral shape must be the interaction between particles and wall and between the particles themselves, while a third friction coefficient is given by the counterflow of air. Clustering is known to occur in bins in the absence of a counterflow of air. The actual values of the friction coefficients (between particles and wall and between the particles themselves) are not of primary importance[4], although they may influence the detailed structure of the spectrum, such as the corner frequencies and the actual slope of the 1/*f* part.

We therefore also examined the spectrum associated with free fall flow out of an open compartment through a gradually narrowing pore (3 mm final diameter) (Fig. 2, curve B). Irregularly shaped, sharp edged steel grit (0.2 mm mean diameter) was used. In both systems the spectrum was white at low frequencies and changed to 1/*f* at higher frequencies. At still higher frequencies the spectrum shape is sensitive to several system parameters and is rather complex.

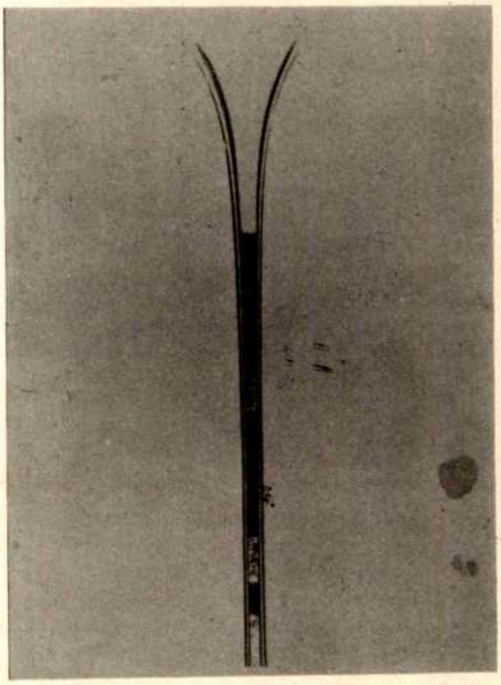

Fig. 1 Flow through hourglass exhibiting clustering (exposure time 1/1000 s).

This is to our knowledge the first report on a physical system with 1/*f* noise which changes at lower frequencies into a different and finite power spectrum. No special tricks need to be invoked, therefore, to circumvent the famous 1/*f* paradox with its explosion of power for frequencies approaching zero[5]. The mechanism at work here might, for this reason, be of general interest, since noise power spectra are investigated in a diversity of fields such as magnetism, superconductivity, semiconductors, artificial and biological membranes and synapses.

Furthermore, the transition from a 1/*f* spectral density into some other spectral density is not the kind predicted by McWhorter[6]; there one must invoke a Poisson process and a distribution of pulse time constants.

It is not very difficult to explain some of the major features of the measured spectra[7]. These include the low frequency form of the power spectrum (white) and the bandwidth over which the spectrum is essentially 1/*f*. Further, it is possible to show, in general, the conditions under which one may expect deviations from a white spectrum and from this obtain important additional insights into possible source mechanisms.

In order to demonstrate these ideas, let us[7], following Heiden[8], view a noise sequence as a sequence of unitary events (pulses) which may represent current, voltage, magnetic lines, and so on. Each individual pulse has its own time constant, τ, amplitude, h, and shape $y(t,\tau)$. In addition, characterising

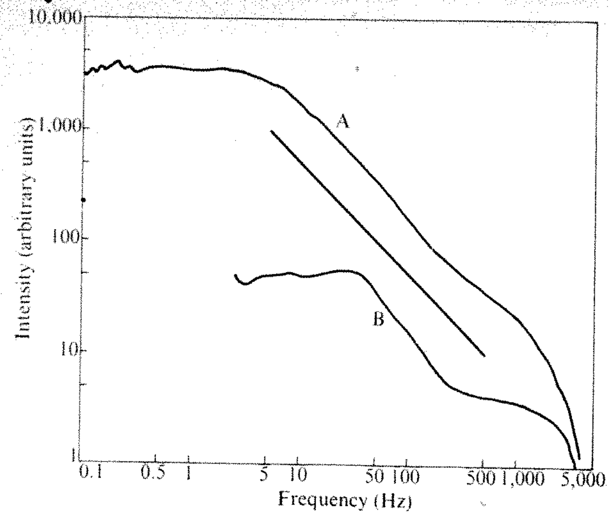

Fig. 2 Power spectra associated with particle flow through hourglass (curve A) and through a narrow pore out of an open compartment (curve B). Line between curves indicates slope of (ideal) $1/f$ noise.

each pulse is the interval φ between its beginning and the beginning of the next pulse. The simplified sequence can be drawn as in Fig. 3.

The interval distribution function $\mu(\varphi)$ may be Poisson or non-Poisson. The sequence can be written as

$$I(t) = h_1 y(t, \tau_1) + h_2 y(t - \varphi_1, \tau_2) + h_3 y(t - \varphi_1 - \varphi_2, \tau_3) + \dots$$

For those sequences in which φ, τ, and h are functionally independent of one another, the power spectrum is[7]

$$S(f) = N\langle h^2\rangle\langle|F(f,\tau)|\rangle^2\left[1 + \frac{2\langle h\rangle^2}{\langle h^2\rangle}\,\frac{|\langle F(f,\tau)\rangle|^2}{\langle|F(f,\tau)|\rangle^2}\,\mathrm{Re}\left(\frac{\psi}{1-\psi}\right)\right]$$

where $\langle\ \rangle$ indicates average value, N is the mean number of pulses per unit time, $F(f,\tau)$ is the Fourier transform of the individual pulse form $y(t,\tau)$, and

$$\psi = \int_0^{\infty} \mu(\varphi)\exp(2\pi i f\varphi)\,d\varphi.$$

For convenience, we can write $S(f) = NA(f)P(f)$ where $A(f) = \langle h^2\rangle\langle|F(f,\tau)|\rangle^2$ and $P(f)$ is the expression in the brackets.

For Poisson distributions, $\mu(\varphi) = N\exp(-N\varphi)$ and $\mathrm{Re}\,(\psi/(1-\psi))$ is zero. In these cases, then, the form of the power spectrum is determined by $F(f,\tau)$ and the distribution of pulse time constants, $\nu(\tau)$.

In general,

$$F(f,\tau) = \int_{-\infty}^{+\infty} y(t,\tau)\exp(-2\pi i f t)\,dt.$$

At low frequencies ($2\pi f\tau \ll 1$), however,

$$F(f,\tau) \cong \int_{-\infty}^{+\infty} y(t,\tau)\,dt.$$

Hence the Fourier transform is frequency independent and the low frequency portion of the spectrum is white[9].

If there is a distribution of time constants, $\nu(\tau)$, among the pulses, then, physically, there exists a maximum τ, τ_m, such that $\nu(\tau)$ is small for $\tau > \tau_m$. Now

$$\langle F(f,\tau)\rangle \simeq \int_0^{\tau_m} F(f,\tau)\,\nu(\tau)\,d\tau,$$

so at frequencies ($2\pi f\tau_m \ll 1$), $\langle F(f,\tau)\rangle$ is also frequency independent, and represents a white spectrum. This is also true for $\langle|F(f,\tau)|\rangle$. Hence, for a sequence of pulses with a distribution of time constants, $A(f)$ is white at low enough frequencies so long as there is no coupling among the parameters of a single pulse.

A similar conclusion obtains for the factor $P(f)$ which incorporates the effect on the power spectrum of deviations from a Poisson sequence.

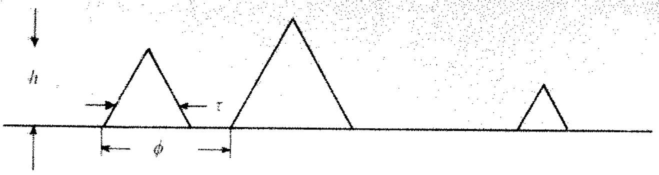

Fig. 3 Pulse sequence parameters.

We have

$$\psi = \int_0^{\infty} \mu(\varphi)\exp(2\pi i f\varphi)\,d\varphi.$$

Physically there exists a maximum φ, say φ_m, beyond which $\mu(\varphi)$ is arbitrarily small. Hence, for low enough frequencies, ($2\pi f\varphi_m \ll 1$), ψ is frequency independent and, therefore, so is $\mathrm{Re}\,(\psi/(1-\psi))$.

We therefore have a theorem which says that the power spectrum of any pulse sequence, Poisson or non-Poisson, even with a distribution of pulse time constants, is white at low enough frequencies provided that there is no coupling among the parameters of a single pulse.

There are at least two immediate and useful consequences. First, if there is not too much overlap between pulses, simple visual inspection of the sequence will permit estimates of τ_m and φ_m. This in turn allows an approximate determination of the low frequency bandwidths over which $A(f)$ and $P(f)$ respectively are white. Hence, under these conditions, visual inspection can tell us whether deviations from a white spectrum are due to $A(f)$ or $P(f)$ over a particular bandwidth. This is particularly easy to do in those cases where $\varphi_m \gg \tau_m$. Secondly, if one should find that at low frequencies ($2\pi f \ll$ the smaller of τ_m^{-1} or φ_m^{-1}) the spectrum is not white, then the implication is that coupling must exist among the parameters of a single pulse.

The first consequence permits direct comparison between the experimentally determined form of the power spectrum and the theoretical frequency range over which $A(f)$ and $P(f)$ should be white. Since one now knows over what bandwidth the form of the spectrum is due to the shape of the individual pulses, we have a direct way of determining if the sequence is non-Poisson (via $P(f)$). The second allows one to deduce that functional relations (for example, $\varphi = (\text{constant})\tau$ or $h = \text{constant}/\varphi$) exist between the pulse parameters. We note for example that $\varphi = (\text{constant})\tau$ is a kind of inhibition where the presence of a pulse with a long time constant tends to delay the appearance of a next pulse while $h = \text{constant}/\varphi$ is a kind of facilitation where a large pulse tends to encourage the appearance of the subsequent pulse. Detailed analysis of the low frequency deviations from a white spectrum will make it possible, in some cases, to select the precise form of the pulse coupling and thereby give further insight into the physical origins of the noise.

If the hourglass flow is examined at different angles with respect to the vertical, the formation of unstable vaults can easily be seen. Clearly, the formation of unstable vaults of different lifetimes generates the clustering effects (Fig. 1) in the flow. In an extensive study (published in Dutch) Peschl[4] investigated the flow of particles from bin openings. A key parameter is the ratio of the opening to the grain diameter. In an hourglass the ratio threshold, 4, is exceeded.

Only unstable vaults occur in normal use and their lifetimes have an upper limit. Hence, the distribution of the intervals between successive particles flowing through a plane must have an upper time limit. In the case of the flow of steel grit through a pore, in our experiments, for example, no intervals longer than 200 ms were found during a period of 10 min with a mean flow of 1,250 grains s^{-1} through the laser beam (0.2 mm diameter). Generally, the individual pulse time constants were considerably less than 20 ms. According to the theorem developed earlier in the paper, the low frequency spectrum should be white for frequencies below about $1/0.2 = 5$ Hz as has been found (Fig. 2). The $1/f$ portion of the spectrum is due to the non-Poisson character (clustering) of the individual particles. At higher frequencies a change from 1/*f* to some other spectrum is predicted due to pulse shape, time constant and photocell response This occurs, as expected, at frequencies ≫ 100 Hz

It is tempting to conjecture that in some systems of molecular dimensions with barriers, particles and pores analogous situations might prevail Molecular size vaults might be formed and thermal motion function as the agent for the introduction of the instabilities These systems would exhibit power spectra with strong low frequency contributions (perhaps 1/*f* in form) but, yet, at the very lowest frequencies their spectra would always turn white

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Second Law of Thermodynamics

IT is widely realised that there is no single and uniquely correct statement of the Second Law of Thermodynamics but rather that there exist a number of different and mutually compatible, correct statements[1] (Everett[2] mentions "two or three dozen") each of which illuminates a different facet of what H A Bent (private communication) has called the "Second Law type of behaviour observed in nature" There is no brief statement from which " all of the thermodynamic relationships "[3] can be deduced without further knowledge In considering a particular problem one particular statement is generally more apposite than another, and for those who are concerned with the mechanisms that produce work, a statement of the Law that stresses the significance of work has distinct advantages One such statement of mine[4] has been criticised by Legon[3] Unfortunately the revised article containing the basis for some of my statements has only recently appeared[5] though it was submitted long before the other[4] This paper[5] should have forestalled many of Legon's objections For example, its first page deals with the question of "maximum work" and its equations (5) to (7) and (9) to (13) deal with equilibria and with entropy creation respectively

Legon's objections are numerous and diverse but I will try to deal systematically with them, as summarised in his last paragraph

Lack of originality. I had not seen the passage quoted from Butler[6] (page 32) when I wrote my paper[4] Legon writes of my statement "All it says is that something that is happening" (that is, that can proceed spontaneously) "can be made to do useful work" Exactly so—it is very simple Taken with its corollary, that unless a proposed change can be made to do useful work it cannot happen spontaneously, the statement specifies conditions both necessary and sufficient for predicting which changes can happen spontaneously and which cannot There is no logical necessity to add any statement about "maximum work" Readers of my two articles[4,5] can surely be in little doubt that I understand at least as well as does Legon the fundamental conclusion of Carnot[7] that to every specified change (isothermal or not) there must correspond a certain definite maximum output of work when going in one direction and an equally definite minimum input of work when going in the opposite one these transfers of work would be realised under reversible conditions, they may or may not be equal depending on the particular system under consideration

I made no claim that my statement of the Second Law sums up the whole of thermodynamics in one sentence neither for that matter does Legon's italicised quotation from Planck[8] (page 103) do so It is necessary to know more of the subject in order to comprehend either statement, and the two can then be seen as not contradictory, but complementary

To understand the 'work' view of the subject in relation to the 'entropy' view of it, one must understand the fact (explicitly stated in refs 4 and 5) that in order to obtain work from a spontaneous process a machine of appropriate design must be introduced, in imagination at least, into the situation This machine constrains the process in such a way that it can occur only if at the same time work is delivered, the machine must also contain a device (for example, a weight) for storing this work since many types of system are not, in themselves, capable of storing work All machines consist of several phases and possess geometrical features, and far from being a mere artifice, the nature of such machines and their limitations are the main topics of interest that thermodynamics has had to offer to many scientists, from the Carnots onwards As Joule[9] (who was interested in muscles as well as in galvanic cells and heat engines) wrote in 1853 "Perhaps the most important applications of dynamical theory are those which refer to the production of motive power from chemical and other actions" The matter could not be more succinctly expressed

Table 1 Range of spontaneous processes

Completely irreversible	Completely reversible
Resulting change in Universe	
Maximum entropy creation	Zero entropy creation
Zero increase in stored work	Max increase in stored work
Max inc in thermal energy	Min inc in thermal energy

Failure to apply to isolated systems. I did not in fact claim[4] that the same equation relating work to entropy creation applied to isolated systems, merely that the "argument can be easily extended" to them, by imagining a machine to be introduced into the system itself This basic idea is far from novel[10] [11] A simple example that illustrates the point well is provided by an isolated system in two parts that are initially at different temperatures Heat is allowed to flow either by ordinary conduction or through a heat engine For a given change of state it is found that the entropy created ΔS_{cr} is always a single-valued function of the work wasted If one part of the system (e) is much larger than the other the equations approach as a limit the simple form given in my paper (ref 5, equation (10))

$$\Delta S_{\mathrm{ci}} = [\text{wasted work}]/T_{\mathrm{e}}$$

Legon's preoccupation with semantics has led him to make heavy weather out of a simple situation Work can certainly be stored in an isolated system—by lifting a weight within the system or in various other ways Revealingly enough, the one form of energy that cannot be used for this purpose is the very one suggested by Legon, that is, thermal energy The fundamental distinction between thermal energy (for definition, see ref 12, page 11) and other types of energy is absolutely basic

Legon poses, as a challenge, the problem of obtaining work from the mixing of two ideal gases in an isolated system of constant total volume It is elementary that if the mixture is allowed to form by merely withdrawing a partition between the gases we have a good example of a completely irreversible process with maximal entropy creation (+11 53 J K^{-1} if we started with 1 mol of each at 300 K) and no performance or storage of work On the other hand, by introducing into the system a suitable machine, the uniform mixture could be allowed to form in such a way that a weight within the system was raised (The machine described by Planck (ref 8, page 219) may be readily adapted for this purpose) At the end of the latter mixing process the isolated system would accordingly contain more mechanical energy than it did at the beginning From the First Law it follows that the system must necessarily contain less thermal energy, that is, its temperature must have fallen In the limit, where the mixing was reversible, the maximum possible work would have been performed and transferred to the weight (2,769 J if the gases were monatomic) and the temperature would have fallen to 189 K In this reversible case the change in entropy arising from mixing (+11 53 J K^{-1}) is exactly counterbalanced by that attributable to cooling (−11 53 J K^{-1}) no entropy is created

At this point it might be objected that the change in the gases is not exactly the same as if they had mixed irreversibly, because their thermal energy and temperature have decreased This is a simple consequence of the First Law which applies equally no matter whether one is considering an isolated system, a non-isolated one or the whole Universe If a change is conducted in such a way that a weight is lifted then all the other bodies involved cannot possibly end up in the same state as if the weight had not been lifted

Failure to apply to nonisothermal systems. Legon expresses doubts about the validity of the equation for entropy creation (refs 3 and 4) save for "the trivial case for which the temperature T_e of the environment is equal to the temperature T of the system throughout the process"[3] On what grounds are these doubts based? Legon does not discuss, let alone dismiss, any of the sources quoted in my article[5] Other relevant sources which should be considered are Keenan and Hatsopoulos[13] and the classic accounts by Maxwell[10] and by Gouy[14]

Legon's quotation from Planck (ref 8, page 104) concerning "dissipated energy" deserves close consideration It seems to state that the maximum work is a definite quantity only for isothermal processes If true this would directly contradict the views of Thomson[15] (later Lord Kelvin) "On a universal tendency in Nature to the dissipation of mechanical energy" On pages 113–117 of ref 8, however, Planck discusses his own statement (ref 8, page 104) and we see that there is in fact no contradiction What Planck demonstrates is that although the change in Helmholtz free energy, $-dA = -d(U-TS)$, measures w_{max} under isothermal conditions, it cannot conveniently be used to determine w_{max} under nonisothermal conditions because the term $S\ dT$ that then appears is frequently indeterminate The same point has already been made in a footnote by Gouy (ref 15, page 506) who had also given the correct equation for determining w_{max} under nonisothermal conditions Accordingly I find no substance in Legon's objections under this heading

If it is thought that there is conflict between the 'work' view of thermodynamics and the 'entropy' view it is high time that the idea was abandoned The two views are different, but symmetrical, aspects of the same reality Spontaneous processes of all kinds fall somewhere within the pattern shown in Table 1, their position depending on the efficiency of the machinery used for the extraction of work

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Information transmission under conditions of sensory shielding

WE present results of experiments suggesting the existence of one or more perceptual modalities through which individuals obtain information about their environment, although this information is not presented to any known sense The literature[1-3] and our observations lead us to conclude that such abilities can be studied under laboratory conditions

We have investigated the ability of certain people to describe graphical material or remote scenes shielded against ordinary perception In addition, we performed pilot studies to determine if electroencephalographic (EEG) recordings might indicate perception of remote happenings even in the absence of correct overt responses

We concentrated on what we consider to be our primary responsibility—to resolve under conditions as unambiguous as possible the basic issue of whether a certain class of paranormal perception phenomena exists So we conducted our experiments with sufficient control, utilising visual, acoustic and electrical shielding, to ensure that all conventional paths of sensory input were blocked At all times we took measures to prevent sensory leakage and to prevent deception, whether intentional or unintentional

Our goal is not just to catalogue interesting events, but to uncover patterns of cause–effect relationships that lend themselves to analysis and hypothesis in the forms with which we are familiar in scientific study The results presented here constitute a first step towards that goal, we have established under known conditions a data base from which departures as a function of physical and psychological variables can be studied in future work

REMOTE PERCEPTION OF GRAPHIC MATERIAL

First, we conducted experiments with Mr Uri Geller in which we examined his ability, while located in an electrically shielded room, to reproduce target pictures drawn by experimenters located at remote locations Second, we conducted double-blind experiments with Mr Pat Price, in which we measured his ability to describe remote outdoor scenes many miles from his physical location Finally, we conducted preliminary tests using EEGs, in which subjects were asked to perceive whether a remote light was flashing, and to determine whether a subject could perceive the presence of the light, even if only at a noncognitive level of awareness

In preliminary testing Geller apparently demonstrated an ability to reproduce simple pictures (line drawings) which had been drawn and placed in opaque sealed envelopes which he was not permitted to handle But since each of the targets was known to at least one experimenter in the room with Geller, it was not possible on the basis of the preliminary testing to discriminate between Geller's direct perception of envelope contents and perception through some mechanism involving the experimenters, whether paranormal or subliminal

So we examined the phenomenon under conditions designed to eliminate all conventional information channels, overt or subliminal Geller was separated from both the target material and anyone knowledgeable of the material, as in the experiments of ref 4

In the first part of the study a series of 13 separate drawing experiments were carried out over 7 days No experiments are deleted from the results presented here

At the beginning of the experiment either Geller or the experimenters entered a shielded room so that from that time forward Geller was at all times visually, acoustically and electrically shielded from personnel and material at the target location Only following Geller's isolation from the experimenters was a target chosen and drawn, a procedure designed to eliminate pre-experiment cueing Furthermore, to eliminate the possibility of pre-experiment target forcing, Geller was kept ignorant as to the identity of the person selecting the target and as to the method of target selection This was accomplished by the use of three different techniques (1) pseudo-random technique of opening a dictionary arbitrarily and choosing the first word that could be drawn (Experiments 1–4), (2) targets, blind to experimenters and subject, prepared independently by SRI scientists outside the experimental group (following Geller's isolation) and provided to the experimenters during the course of the experiment (Experiments 5–7, 11–13), and (3) arbitrary selection from a target pool decided upon in advance of daily experimentation and designed to provide data concerning information content for use in testing specific hypotheses (Experiments 8–10). Geller's task was to reproduce with pen on paper the line drawing generated at the target location Following a period of effort ranging from a few minutes to half an hour, Geller either passed (when he did not feel confident) or indicated he was ready to submit a drawing to the experimenters, in which case the drawing was collected before Geller was permitted to see the target

To prevent sensory cueing of the target information, Experiments 1 through 10 were carried out using a shielded room in SRI's facility for EEG research The acoustic and visual isolation is provided by a double-walled steel room, locked by means of an inner and outer door, each of which is secured with a refrigerator-type locking mechanism Following target selection when Geller was inside the room, a one-way audio monitor, operating only from the inside to the outside, was activated to monitor Geller during his efforts The target picture was never discussed by the experimenters after the picture was drawn and brought near the shielded room In our detailed examination of the shielded room and the protocol used in these experiments, no sensory leakage has been found

The conditions and results for the 10 experiments carried out in the shielded room are displayed in Table 1 and Fig 1 All experiments except 4 and 5, were conducted with Geller inside the shielded room In Experiments 4 and 5, the procedure was reversed For those experiments in which Geller was inside the shielded room, the target location was in an adjacent room at a distance of about 4 m, except for Experiments 3 and 8, in which the target locations were, respectively, an office at a distance of 475 m and a room at a distance of about 7 m

A response was obtained in all experiments except Numbers 5–7 In Experiment 5, the person-to-person link was eliminated by arranging for a scientist outside the usual experimental group to draw a picture, lock it in the shielded room before Geller's arrival at SRI, and leave the area Geller was then led

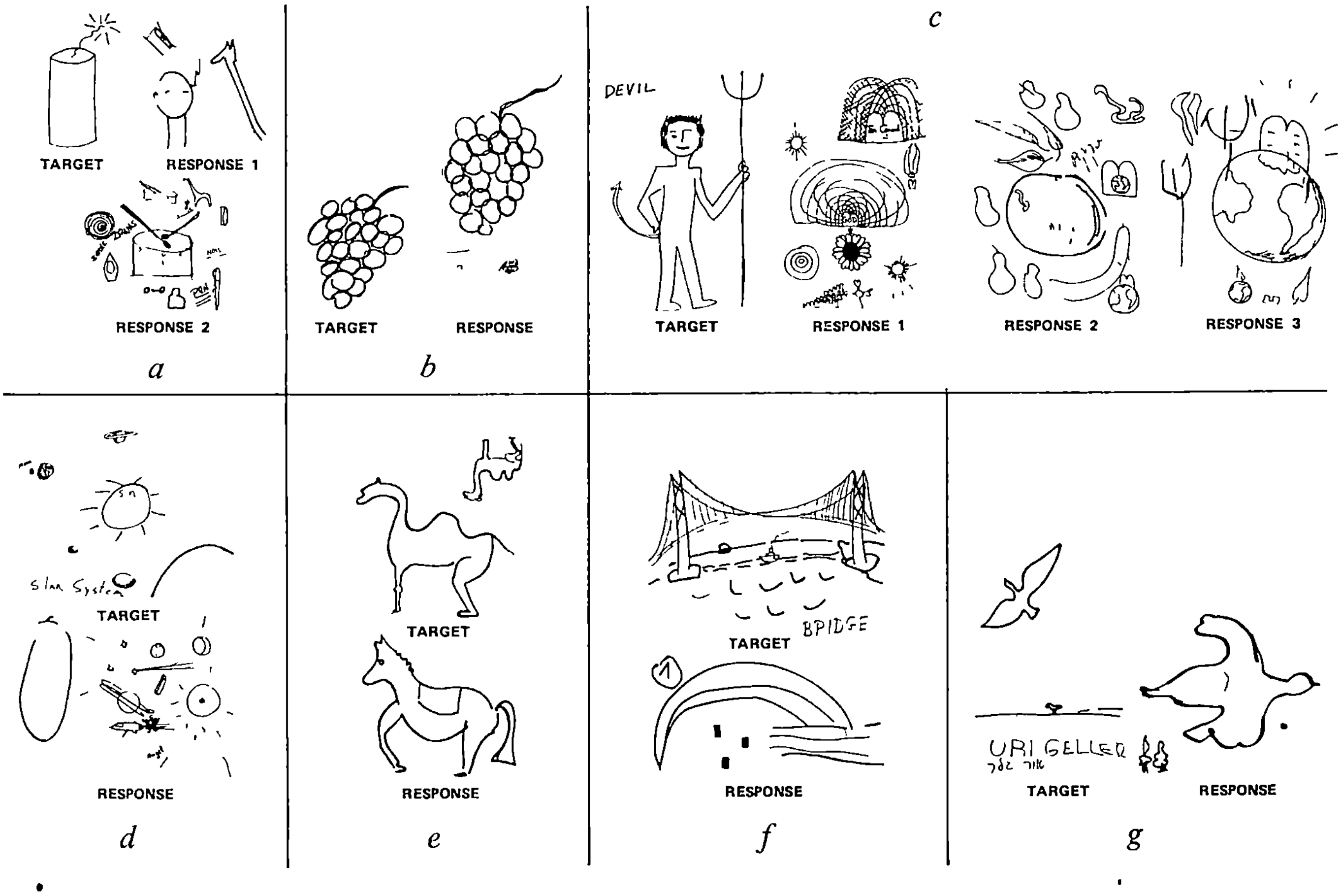

Fig. 1 Target pictures and responses drawn by Uri Geller under shielded conditions

Table 1 Remote perception of graphic material

Experiment	Date (month, day, year)	Geller Location	Target Location	Target	Figure
1	8/4/73	Shielded room 1*	Adjacent room (4 1 m)†	Firecracker	1*a*
2	8/4/73	Shielded room 1	Adjacent room (4 1 m)	Grapes	1*b*
3	8/5/73	Shielded room 1	Office (475 m)	Devil	1*c*
4	8/5/73	Room adjacent to shielded room 1	Shielded room 1 (3 2 m)	Solar system	1*d*
5	8/6/73	Room adjacent to shielded room 1	Shielded room 1 (3 2 m)	Rabbit	No drawing
6	8/7/73	Shielded room 1	Adjacent room (4 1 m)	Tree	No drawing
7	8/7/73	Shielded room 1	Adjacent room (4 1 m)	Envelope	No drawing
8	8/8/73	Shielded room 1	Remote room (6 75 m)	Camel	1*e*
9	8/8/73	Shielded room 1	Adjacent room (4 1 m)	Bridge	1*f*
10	8/8/73	Shielded room 1	Adjacent room (4 1 m)	Seagull	1*g*
11	8/9/73	Shielded room 2‡	Computer (54 m)	Kite (computer CRT)	2*a*
12	8/10/73	Shielded room 2	Computer (54 m)	Church (computer memory)	2*b*
13	8/10/73	Shielded room 2	Computer (54 m)	Arrow through heart (computer CRT, zero intensity)	2*c*

*EEG Facility shielded room (see text)
†Perceiver–target distances measured in metres
‡SRI Radio Systems Laboratory shielded room (see text)

by the experimenters to the shielded room and asked to draw the picture located inside the room He said that he got no clear impression and therefore did not submit a drawing The elimination of the person-to-person link was examined further in the second series of experiments with this subject

Experiments 6 and 7 were carried out while we attempted to record Geller's EEG during his efforts to perceive the target pictures The target pictures were, respectively, a tree and an envelope He found it difficult to hold adequately still for good EEG records, said that he experienced difficulty in getting impressions of the targets and again submitted no drawings

Experiments 11 through 13 were carried out in SRI's Engineering Building, to make use of the computer facilities available there For these experimenters, Geller was secured in a double-walled, copper-screen Faraday cage 54 m down the hall and around the corner from the computer room The Faraday cage provides 120 dB attenuation for plane wave radio frequency radiation over a range of 15 kHz to 1 GHz For magnetic fields the attenuation is 68 dB at 15 kHz and decreases to 3 dB at 60 Hz Following Geller's isolation, the targets for these experiments were chosen by computer laboratory personnel not otherwise associated with either the experiment or Geller, and the experimenters and subject were kept blind as to the contents of the target pool

For Experiment 11, a picture of a kite was drawn on the face of a cathode ray tube display screen, driven by the computer's graphics program For Experiment 12, a picture of a church was drawn and stored in the memory of the computer In Experiment 13, the target drawing, an arrow through a heart (Fig 2*c*), was drawn on the face of the cathode ray tube and then the display intensity was turned off so that no picture was visible

To obtain an independent evaluation of the correlation between target and response data, the experimenters submitted the data for judging on a 'blind' basis by two SRI scientists who were not otherwise associated with the research For the 10 cases in which Geller provided a response, the judges were asked to match the response data with the corresponding target data (without replacement) In those cases in which Geller made more than one drawing as his response to the target, all the drawings were combined as a set for judging The two judges each matched the target data to the response data with no error For either judge such a correspondence has an *a priori* probability, under the null hypothesis of no information channel, of $P = (10!)^{-1} = 3 \times 10^{-7}$

A second series of experiments was carried out to determine whether direct perception of envelope contents was possible without some person knowing of the target picture

One hundred target pictures of everyday objects were drawn by an SRI artist and sealed by other SRI personnel in double envelopes containing black cardboard The hundred targets were divided randomly into groups of 20 for use in each of the three days' experiments

On each of the three days of these experiments, Geller passed That is, he declined to associate any envelope with a drawing that he made, expressing dissatisfaction with the existence of such a large target pool On each day he made approximately 12 recognisable drawings, which he felt were associated with the entire target pool of 100 On each of the three days, two of his drawings could reasonably be associated with two of the 20 daily targets On the third day, two of his drawings were very close replications of two of that day's target pictures The drawings resulting from this experiment do not depart significantly from what would be expected by chance

In a simpler experiment Geller was successful in obtaining information under conditions in which no persons were knowledgeable of the target A double-blind experiment was performed in which a single 3/4 inch die was placed in a 3 × 4 × 5 inch steel box The box was then vigorously shaken by one of the experimenters and placed on the table, a technique found in control runs to produce a distribution of die faces differing nonsignificantly from chance The orientation of the die within the box was unknown to the experimenters at that time Geller would then write down which die face was uppermost The target pool was known, but the targets were individually prepared in a manner blind to all persons involved in the experiment This experiment was performed ten times, with Geller passing twice and giving a response eight times In the eight times in which he gave a response, he was correct each time The distribution of responses consisted of three 2s, one 4, two 5s, and two 6s The probability of this occurring by chance is approximately one in 10^6

In certain situations significant information transmission can take place under shielded conditions Factors which appear to be important and therefore candidates for future investigation include whether the subject knows the set of targets in the target pool, the actual number of targets in the target pool at any given time, and whether the target is known by any of the experimenters

It has been widely reported that Geller has demonstrated the ability to bend metal by paranormal means Although metal bending by Geller has been observed in our laboratory, we have not been able to combine such observations with adequately controlled experiments to obtain data sufficient to support the paranormal hypothesis

REMOTE VIEWING OF NATURAL TARGETS

A study by Osis[5] led us to determine whether a subject could describe randomly chosen geographical sites located several miles from the subject's position and demarcated by some appropriate means (remote viewing) This experiment carried out with Price, a former California police commissioner and city councilman, consisted of a series of double-blind, demonstration-of-ability tests involving local targets in the San Francisco Bay area which could be documented by several independent judges We planned the experiment considering that natural geographical places or man-made sites that have existed for a long time are more potent targets for paranormal perception experiments than are artificial targets prepared in the laboratory This is based on subject opinions that the use of artificial targets involves a 'trivialisation of the ability' as compared with natural pre-existing targets

In each of nine experiments involving Price as subject and SRI experimenters as a target demarcation team, a remote location was chosen in a double-blind protocol Price, who remained at SRI, was asked to describe this remote location, as well as whatever activities might be going on there

Several descriptions yielded significantly correct data pertaining to and descriptive of the target location

In the experiments a set of twelve target locations clearly differentiated from each other and within 30 min driving time from SRI had been chosen from a target-rich environment (more than 100 targets of the type used in the experimental series) prior to the experimental series by an individual in SRI management, the director of the Information Science and Engineering Division, not otherwise associated with the experiment Both the experimenters and the subject were kept blind as to the contents of the target pool, which were used without replacement

An experimenter was closeted with Price at SRI to wait 30 min to begin the narrative description of the remote location The SRI locations from which the subject viewed the remote locations consisted of an outdoor park (Experiments 1, 2), the double-walled copper-screen Faraday cage discussed earlier (Experiments 3, 4, and 6–9), and an office (Experiment 5) A second experimenter would then obtain a target location from the Division Director from a set of travelling orders previously prepared and randomised by the Director and kept under his control The target demarcation team (two to four SRI experimenters) then proceeded directly to the target by automobile without communicating with the subject or experimenter remaining behind Since the experimenter remaining with the subject at SRI was in ignorance both as to the particular target and as to the target pool, he was free to question Price to clarify his descriptions The demarcation team then remained at the target site for 30 min after the 30 min allotted for travel During the observation period, the remote-viewing subject would describe his impressions of the target site into a tape recorder A comparison was then made when the demarcation team returned

Price's ability to describe correctly buildings, docks, roads, gardens and so on, including structural materials, colour, ambience and activity, sometimes in great detail, indicated the functioning of a remote perceptual ability But the descriptions contained inaccuracies as well as correct statements To obtain a numerical evaluation of the accuracy of the remote viewing experiment, the experimental results were subjected to independent judging on a blind basis by five SRI scientists who were

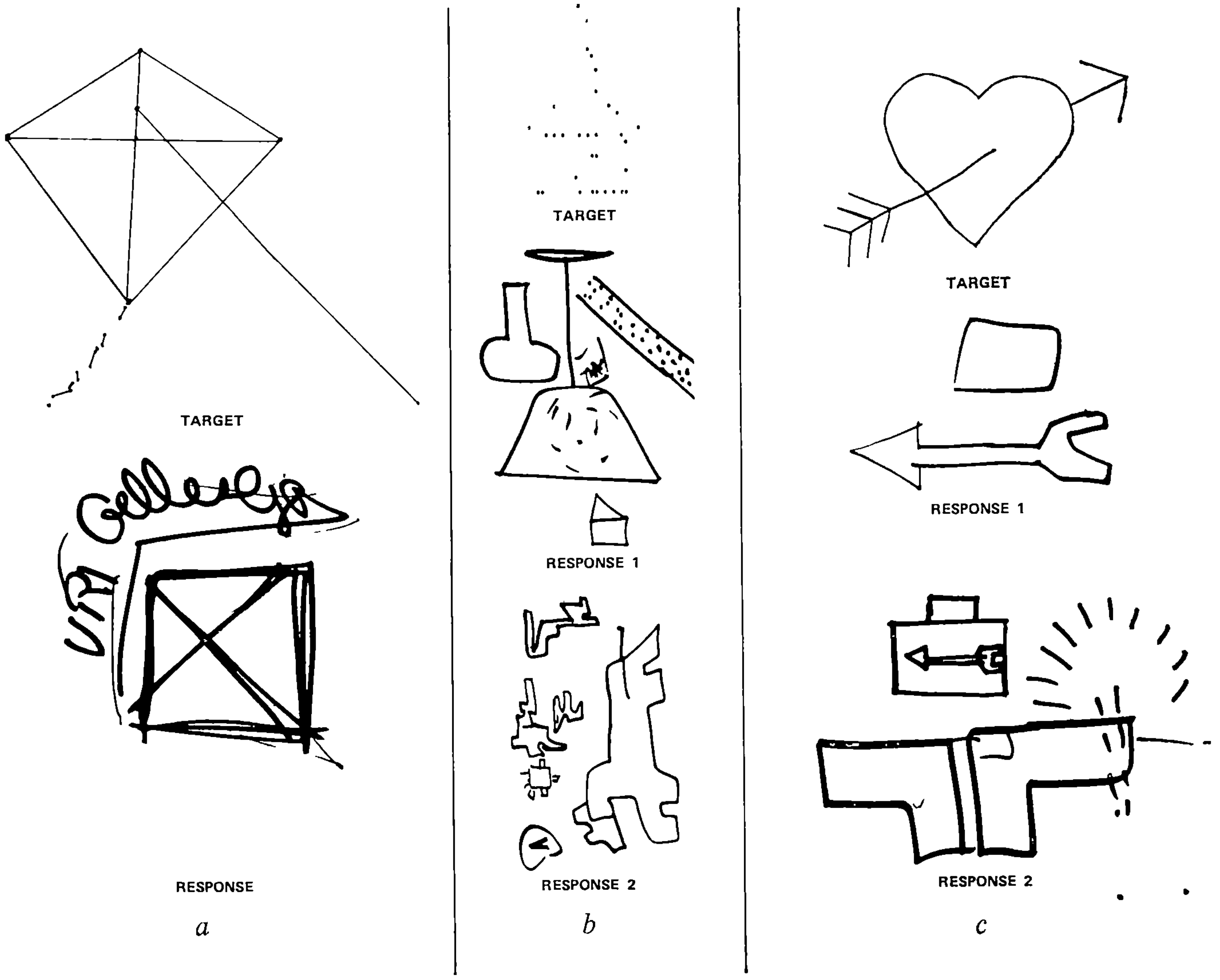

Fig 2 Computer drawings and responses drawn by Uri Geller a, Computer drawing stored on video display, b, computer drawing stored in computer memory only, c, computer drawing stored on video display with zero intensity

Table 2 Distribution of correct selections by judges A, B, C, D, and E in remote viewing experiments

Descriptions chosen by judges		Places visited by judges 1	2	3	4	5	6	7	8	9
Hoover Tower	1	ABCDE				D				
Baylands Nature Preserve	2		ABC	E				D		D
Radio Telescope	3			ACD		BE				
Redwood City Marina	4		CD		ABDE		E			
Bridge Toll Plaza	5						ABD		DCE	
Drive-In Theatre	6			B		A	C			E
Arts and Crafts Garden Plaza	7							ABCE		
Church	8				C				AB	
Rinconada Park	9		CE							AB

Of the 45 selections (5 judges, 9 choices), 24 were correct Bold type indicates the description chosen most often for each place visited Correct choices lie on the main diagonal The number of correct matches by Judges A through E is 7, 6, 5, 3, and 3, respectively The expected number of correct matches from the five judges was five, in the experiment 24 such matches were obtained The *a priori* probability of such an occurrence by chance, conservatively assuming assignment without replacement on the part of the judges, is $P = 8\ 10^{-10}$

not otherwise associated with the research. The judges were asked to match the nine locations, which they independently visited, against the typed manuscripts of the tape-recorded narratives of the remote viewer The transcripts were unlabelled and presented in random order The judges were asked to find a narrative which they would consider the best match for each of the places they visited A given narrative could be assigned to more than one target location A correct match requires that the transcript of a given date be associated with the target of that date Table 2 shows the distribution of the judges' choices

Among all possible analyses, the most conservative is a permutation analysis of the plurality vote of the judges' selections assuming assignment without replacement, an approach independent of the number of judges By plurality vote, six of the nine descriptions and locations were correctly matched Under the null hypothesis (no remote viewing and a random selection of descriptions without replacement), this outcome has an *a priori* probability of $P = 5\ 6 \times 10^{-4}$, since, among all possible permutations of the integers one through nine, the probability of six or more being in their natural position in the list has that value Therefore, although Price's descriptions contain inaccuracies, the descriptions are sufficiently accurate to permit the judges to differentiate among the various targets to the degree indicated

EEG EXPERIMENTS

An experiment was undertaken to determine whether a physiological measure such as EEG activity could be used as an indicator of information transmission between an isolated subject and a remote stimulus We hypothesised that perception could be indicated by such a measure even in the absence of verbal or other overt indicators[6,7]

It was assumed that the application of remote stimuli would result in responses similar to those obtained under conditions of direct stimulation For example, when normal subjects are stimulated with a flashing light, their EEG typically shows a decrease in the amplitude of the resting rhythm and a driving of the brain waves at the frequency of the flashes[8] We hypothesised that if we stimulated one subject in this manner (a sender), the EEG of another subject in a remote room with no flash present (a receiver), might show changes in alpha (9–11 Hz) activity, and possibly EEG driving similar to that of the sender

We informed our subject that at certain times a light was to be flashed in a sender's eyes in a distant room, and if the subject perceived that event, consciously or unconsciously, it might be evident from changes in his EEG output The receiver was seated in the visually opaque, acoustically and electrically shielded double-walled steel room previously described The sender was seated in a room about 7 m from the receiver

To find subjects who were responsive to such a remote stimulus, we initially worked with four female and two male volunteer subjects, all of whom believed that success in the experimental situation might be possible These were designated 'receivers' The senders were either other subjects or the experimenters We decided beforehand to run one or two sessions of 36 trials each with each subject in this selection procedure, and to do a more extensive study with any subject whose results were positive

A Grass PS-2 photostimulator placed about 1 m in front of the sender was used to present flash trains of 10 s duration The receiver's EEG activity from the occipital region (O_z), referenced to linked mastoids, was amplified with a Grass 5P-1 preamplifier and associated driver amplifier with a bandpass of 1–120 Hz The EEG data were recorded on magnetic tape with an Ampex SP 300 recorder

On each trial, a tone burst of fixed frequency was presented to both sender and receiver and was followed in one second by either a 10 s train of flashes or a null flash interval presented to the sender Thirty-six such trials were given in an experimental session, consisting of 12 null trials—no flashes following the tone—12 trials of flashes at 6 f p s and 12 trials of flashes at 16 f p s , all randomly intermixed, determined by entries from a table of random numbers Each of the trials generated an 11-s EEG epoch The last 4 s of the epoch was selected for analysis to minimise the desynchronising action of the warning cue This 4-s segment was subjected to Fourier analysis on a LINC 8 computer

Spectrum analyses gave no evidence of EEG driving in any receiver, although in control runs the receivers did exhibit driving when physically stimulated with the flashes But of the six subjects studied initially, one subject (H H) showed a consistent alpha blocking effect We therefore undertook further study with this subject

Data from seven sets of 36 trials each were collected from this subject on three separate days This comprises all the data collected to date with this subject under the test conditions described above The alpha band was identified from average spectra, then scores of average power and peak power were obtained from individual trials and subjected to statistical analysis

Of our six subjects, H H had by far the most monochromatic EEG spectrum Figure 3 shows an overlay of the three averaged spectra from one of this subject's 36-trial runs, displaying changes in her alpha activity for the three stimulus conditions

Mean values for the average power and peak power for each

Table 3 EEG data for H H showing average power and peak power in the 9–11 Hz band, as a function of flash frequency and sender

Flash Frequency	0	6	16	0	6	16
Sender	Average Power			Peak Power		
J L	94 8	84 1	76 8	357 7	329 2	289 6
R T	41 3	45 5	37 0	160 7	161 0	125 0
No sender (subject informed)	25 1	35 7	28 2	87 5	95 7	81 7
J L	54 2	55 3	44 8	191 4	170 5	149 3
J.L	56 8	50 9	32 8	240 6	178 0	104 6
R T	39 8	24 9	30 3	145 2	74 2	122 1
No sender (subject not informed)	86 0	53 0	52 1	318 1	180 6	202 3
Averages	56 8	49 9	43 1	214 5	169 8	153 5
		−12%	−24%($P<0\ 04$)		−21%	−28%($P<0\ 03$)

Each entry is an average over 12 trials

of the seven experimental sets are given in Table 3 The power measures were less in the 16 f p s case than in the 0 f p s in all seven peak power measures and in six out of seven average power measures Note also the reduced effect in the case in which the subject was informed that no sender was present (Run 3) It seems that overall alpha production was reduced for this run in conjunction with the subject's expressed apprehension about conducting the experiment without a sender This is in contrast to the case (Run 7) in which the subject was not informed

Siegel's two-tailed t approximation to the nonparametric randomisation test[9] was applied to the data from all sets, which included two sessions in which the sender was removed Average power on trials associated with the occurrence of 16 f p s was significantly less than when there were no flashes ($t = 2\,09$, d f $= 118$, $P < 0\,04$) The second measure, peak power, was also significantly less in the 16 f p s conditions than in the null condition ($t = 2\,16$, d f $= 118$, $P < 0\,03$) The average response in the 6 f p s condition was in the same direction as that associated with 16 f p s , but the effect was not statistically significant

Spectrum analyses of control recordings made from saline with a 12 kΩ resistance in place of the subject with and without the addition of a 10 Hz, 50 μV test signal applied to the saline solution, revealed no indications of flash frequencies, nor perturbations of the 10 Hz signal These controls suggest that the results were not due to system artefacts Further tests also gave no evidence of radio frequency energy associated with the stimulus

Subjects were asked to indicate their conscious assessment for each trial as to which stimulus was generated They made their guesses known to the experimenter via one-way telegraphic communication An analysis of these guesses has shown them to be at chance, indicating the absence of any supraliminal cueing, so arousal as evidenced by significant alpha blocking occurred only at the noncognitive level of awareness

We hypothesise that the protocol described here may prove to be useful as a screening procedure for latent remote perceptual ability in the general population

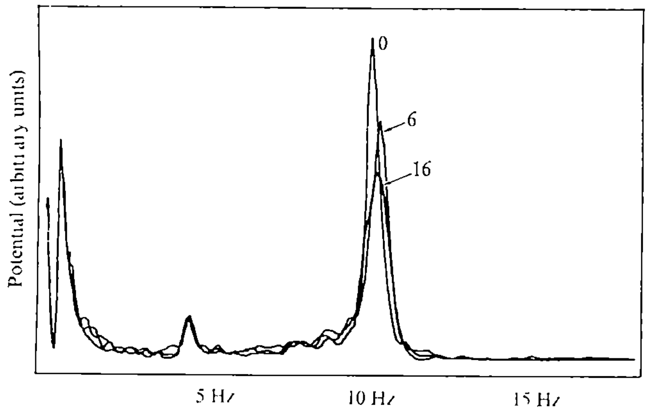

Fig 3 Occipital EEG spectra, 0–20 Hz, for one subject (H H) acting as receiver, showing amplitude changes in the 9–11 Hz band as a function of strobe frequency Three cases 0, 6, and 16 f p s (12 trial averages)

CONCLUSION

From these experiments we conclude that

- A channel exists whereby information about a remote location can be obtained by means of an as yet unidentified perceptual modality
- As with all biological systems, the information channel appears to be imperfect, containing noise along with the signal
- While a quantitative signal-to-noise ratio in the information-theoretical sense cannot as yet be determined, the results of our experiments indicate that the functioning is at the level of useful information transfer

It may be that remote perceptual ability is widely distributed in the general population, but because the perception is generally below an individual's level of awareness, it is repressed or not noticed For example, two of our subjects (H H and P P) had not considered themselves to have unusual perceptual ability before their participation in these experiments

Our observation of the phenomena leads us to conclude that experiments in the area of so-called paranormal phenomena can be scientifically conducted, and it is our hope that other laboratories will initiate additional research to attempt to replicate these findings

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The stability of a feasible random ecosystem

THE weight of the evidence, and the beliefs of most biologists, seem to support the view[1] that ecosystems tend to be more stable, the larger the number of interacting species they contain It is puzzling, therefore, that a variety of mathematical models of complex ecosystems appear to give the contrary answer that complexity makes for instability[2]

Prominent among such models is the complex system with random interactions, studied in various forms by Gardner and Ashby[3] and May[4], but their results cannot be applied as they stand to ecological systems In an ecosystem, the interacting variables are species populations (or species biomass) which cannot take on negative values Thus, for example, the equilibrium population values must be positive, and it is convenient to denote this necessary property of an ecosystem model by saying that it must be 'feasible'

The work referred to imposed no such constraint on equilibrium populations in the samples considered It is of some interest, therefore, to examine the stability of a random model capable of representing ecosystems, by imposing the restriction that the sample be feasible

I report here the results of computer calculations on such a model The interaction equations were of the well-known quasi-linear type, in which the rate of fractional increase of a species population is a linear function of the current populations in all T species That is, the number N_i in the ith species obeys

$$dN_i/dt = N_i\,(b_i + \Sigma_j\, a_{ij}\, N_j)$$

All birth rates b_i were taken as 1, and the self-regulating coefficients a_{ii} as -1 The feasibility requirement was that the

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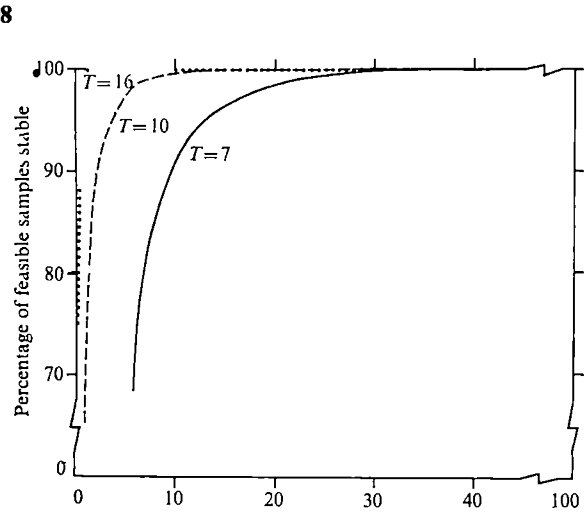

Fig 1 Stability of feasible samples

solutions for the equilibrium populations N^*_i, obtained by equating the dN_i/dt to zero, should be strictly positive. The stability matrix S is given by $S_{ij} = N^*_i\, a_{ij}$, S is unstable if it has an eigenvalue with positive real part. The interaction coefficients were chosen to have a fixed magnitude z but random sign (+ or − equally likely), with all T species uniformly self regulated. A minimum of 2,000 samples were examined for each value of T and z.

When all samples were accepted—whether feasible or not—the results for $T = 7$, 10 and 16 agreed qualitatively with the related (though not identical) models of refs 3 and 4. That is, for z small, essentially all samples were stable, but the stable fraction tended to zero as z approached $T^{-\frac{1}{2}}$.

On examining the stability of the feasible samples only, however, a significantly different behaviour emerges. We see from Fig 1 that feasible samples are generally stable, the stable percentage departing from close to 100% only when the occurrence of a feasible sample is in any case rare (that is, when z is large). Moreover, this tendency to stability increases with the number T of interacting species. (It should be noted that the curves in Fig 1 contain no extrapolated portions, but are constructed by interpolating between computed points of negligible Monte Carlo error.)

The results displayed suggest the conclusion that, for this model type, almost all feasible systems are stable when the number of interacting species increases without limit. (The curve for $T = 16$ is already barely distinguishable from such a step function.)

The results confirm previous findings (both numerical[3] and analytical[4]), in showing that both feasibility and stability are rare when $z > T^{-\frac{1}{2}}$. It is not unreal, however, to expect average interaction strength to decrease as the number of interactions increases.

It is easy to see why the general conclusions emerging differ from those of refs 3 and 4, when these are specialised to ecosystems. The latter studies worked directly on the stability matrix, and thus could not impose any feasibility requirement (this must be derived from the solutions of the original interaction equations, which do not appear in their approach). Now, unfeasible samples contain at least one species with a negative equilibrium population, this simply means, biologically, that after a time the species becomes extinct. But, if this extinction is ignored, such 'ghost species' are being implicitly retained in the stability examination and exert a profoundly destabilising effect. In the quasi-linear model above, for example, they 'live on' as an unregulated, exponentially increasing component of the system. The diagonal element of the stability matrix is $-N^*_i$, which is positive when $N^*_i < 0$. Thus the ghost species figures in it like a real species (that is, one with a positive number at equilibrium) with $a_{ii} = +1$, that is, with an eventual population increase proportional to N_i^2.

The results cited above show that, when these 'mathematical ghosts' are excluded, the model shows an actual increase of stability in the larger systems.

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Objective evaluation of auditory evoked EEG responses

THE averaging of often very small responses is widely used in physiology, psychology and clinical medicine to evaluate stimulus threshold levels. It is sometimes difficult to recognise responses in the average waveform because of residual activity, so a means of improving sensitivity and objectively recognising positive responses would be useful. We have previously found[1] that the averaged responses (ERA) evoked in the electroencephalogram (EEG) of a subject by auditory stimuli were produced by imposing a phase constraint on the different Fourier harmonics of the spontaneous EEG, without necessarily influencing their amplitudes.

Merely imposing the phase-spectrum characteristic of a response to high-level stimuli on length of unstimulated spontaneous EEG from the same or another individual, creates a waveform similar to and highly correlated with a true average of responses to high-level stimuli and a similar result is produced by imposing the appropriate phase spectrum on lengths of pseudo-random white Gaussian noise instead of on spontaneous EEG. These observations provide a convenient basis for the objective detection of the existence of positive responses to given stimuli and for the determination of auditory threshold levels.

Our previous findings concerned the average, rather than the individual, evoked response but clearly must reflect phenomena in the separate lengths of EEG following individual stimuli. If so, it should be possible to demonstrate a phase-constraint operative on the various harmonic components of the individual post-stimulus lengths of EEG and such a demonstration would be useful in suggesting the basis of an objective assessment of the occurrence of response. Unfortunately, phase spectra do present difficulty of interpretation and in the present context, may be liable to some error if unnecessarily long record lengths are used. One problem is that phase values are restricted to the range −180° to +180°, and as a result of the repeated recurrence of the principal range of angles that can occur, averaging phase-spectral values for any given harmonic component, in successive sweeps, is usually unsatisfactory. We have therefore investigated instead whether the ensemble distribution of phase values for each harmonic component shows any aggregation, or if it shows the uniform distribution characteristic of uncontrolled phases as seen in unstimulated records.

Phase values have been calculated for each of the first ten Fourier harmonic components and classified into four equal-size groups covering the full 360° range of possible values. The null hypothesis leads to a uniform distribution of phase values so that for 60 sweeps, the expected number of values is 15 in each class. A χ^2 test with d f = 3 can then be applied to test the null hypothesis (that is, no phase control), for each harmonic separately The results show that for high-level stimuli, the null hypothesis must be rejected on the basis of one or more harmonics ($P<0$ 01), but for stimuli that are evidently sub-threshold according to both subjective judgment and appearance of the averaged response waveform, the null hypothesis holds Intermediate levels lead to χ^2 values that broadly decrease in significance as stimulus level decreases and the 5% level occurs near the subjective threshold For high-level stimuli which produce an evident response, most of the harmonics exhibit the phase constraint, but as the stimulus level is reduced towards threshold, significant aggregation of phase values is confined only to a few harmonics The effects reported here have been studied for increasing lengths of post stimulus record, starting with about 0 32 s and ultimately reaching the full length of our records (0 94 s post stimulus) The existence of a phase constraint is increasing evident with increasing record length, the effect often fails altogether, however, when the record length is allowed to exceed about 0 8 s This observation suggests that after about 0 75 s any phase control of spontaneous EEG activity diminishes, allowing fully spontaneous conditions to be generally restored beyond this point If the record used for analysis did include, for this reason, a segment of spontaneous activity, the appearance of the phase constraint would then be lost

These findings apply in each of the trials with different tonal or click stimuli for all the six subjects (three adults, three children) reported previously[1], and similarly to each of the records from three further subjects that we have so far examined Thus, a non-subjective and quantitative system of auditory assessment would seem to be feasible, utilising phase-spectral measurements alone This approach, however, implicitly utilises some information about the amplitude spectrum of the signal, in that low amplitude Fourier components are neglected (by restricting analysis to the first ten or fewer harmonics), and we would not rule out the possibility of utilising further amplitude spectral information to supplement the indications provided by a phase analysis

We would also remark that the characterisation of these signals in phase-spectral terms is an abstraction to describe a mechanism that is perhaps more realistically envisaged as a synchronisation process in the time domain, rather than as a phase-constraint in the frequency domain

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Imprinting and exploration of slight novelty in chicks

Shortly after hatching, domestic chicks will respond socially to a wide range of moving or conspicuous objects As a result of 'imprinting', their social behaviour is increasingly limited to objects with which they have had visual experience In general, the longer that chicks have been exposed to an object, the more strongly they prefer it when given a choice between it and something else[1-5] Some evidence, however, suggests that in the early stages of imprinting, the trend towards a highly restricted preference is temporarily reversed and some birds may even prefer the unfamiliar object[6,7]

Here we report that day-old chicks will even work to expose themselves to novel stimulation at an early stage in imprinting At that stage, by changing the characteristics of a visual stimulus when a chick stepped onto a pedal, it was possible to increase the likelihood that it would step on to the pedal again

Bateson and Reese[8] found that day-old chicks hatched and reared in the dark would rapidly learn to press a pedal which turned on a flashing rotating light We have used a similar technique in this study but with two important differences first, the chicks had been exposed to a flashing, rotating light for varying amounts of time before the experiment, second, the familiar stimulus was turned on when a bird was not on the pedal As soon as the chick stepped on the pedal the colour of the stimulus was changed In other words, the bird was exposed to a novel stimulus for as long as it stood on the pedal

The apparatus consisted of a chamber measuring 37 5 by 34 5 by 33 cm Two of the walls were made of hardboard and the front and back walls of 1 27-cm wire mesh Two pedals were set into the floor, each measured 4 8 cm square and was placed 2 0 cm from the wire mesh front and 2 6 cm from one of the sides All the apparatus was painted matt black except for the pedals which were matt white The temperature 5 cm above the floor of the apparatus was 23 5–26 5° C The stimulus was placed 60 cm in front of the wire mesh and midway between the two pedals It

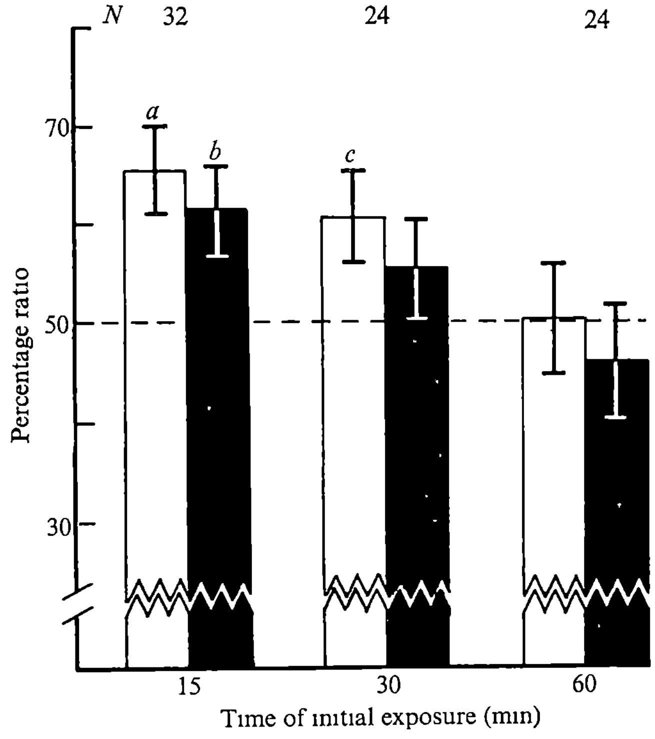

Fig 1 Means and s e for the total presses by day-old chicks of a pedal altering a flashing light from a familiar to unfamiliar colour The pedal presses are expressed as percentages of the total presses of that pedal and another symetrically placed pedal having no effect on the light Open bars are percentage durations of pressing and solid bars are percentage frequencies of pressing The chicks were previously exposed to the familiar light for 15, 30 or 60 min Departures from the chance level of 50% are indicated as follows a, $t=3$ 29, $P<0$ 01, b, $t=2$ 56, $P<0$ 02, c, $t=2$ 14, $P<0$ 05

consisted of a translucent cylinder 15 3 cm in diameter and 16 cm in length rotating at 45 r p m on a horizontal axis The cylinder was covered with a chequerboard pattern (2-cm squares) A primary red and primary yellow filter were fitted inside the cylinder, each over a 45 W bulb The bulbs could be individually lit to make the whole cylinder appear as either red or yellow checks, the background was matt black When one pedal was pressed the colour of the cylinder changed, the other pedal had no effect on the stimulus and was used to measure any general changes in activity

Eighty Ross 'Chunky' chicks were hatched in the laboratory and individually exposed to constant white light for 50 min When they were 14–22 h old, half were individually exposed in activity wheels[9] to a flashing rotating light like the red stimulus in the operant conditioning situation and half were exposed to one like the yellow stimulus The chicks were exposed for 15, 30 or 60 min and 3 min later were separately placed in the operant conditioning box The experimental design was balanced so that for half the chicks the pedal changing the colour of the stimulus was on the right hand side and for the other half it was on the left hand side Each chick was left in the apparatus for 50 min

The results are shown in Fig 1 The presses of the pedal changing the colour of the light are expressed as a percentage of the total presses of both pedals, if they were responding at the chance level the ratio would have been 50% The absolute measures of pedal pressing were the same for all groups In Fig 1 percentages are given for both the number of presses in the 50-min tests and the total time spent pressing the pedals It can be seen that after 15 min previous experience with the familiar stimulus the chicks pressed the pedal which changed the colour of the light more frequently and for longer than they did the other pedal The red-trained birds did not differ from the yellow-trained birds in the extent to which they worked for change In the red-trained chicks exposed for 30 min, the effect was much less clearly marked and by 60 min the birds in both groups were responding at the chance level The overall decline in the ratios is statistically significant for both measures (for response number Pearson $r=-0\ 242$, $P<0\ 05$, for response duration, $r=-0\ 240$, $P<0\ 05$)

Differential pressing of the two pedals cannot be attributed to an initial preference for one stimulus over the other regardless of previous experience because the experimental design was balanced and the subgroups did not differ Similarly their behaviour cannot be explained in terms of a preference for the position of one pedal over the other We believe that the results unequivocally support earlier suggestions that chicks prefer novel objects at an early stage in imprinting[6] Moreover, they show that the birds will actively work to expose themselves to slightly novel stimulation The biological advantages of such behaviour may lie in the very complexity of what the young bird has to learn under natural conditions The ability to recognise a parent from all likely angles almost certainly requires more information than could possibly be acquired as the result of learning the characteristics of a single view of that parent The transformation from front view to side view and from side view to back view could not easily be accomplished by mechanisms ordinarily understood as being involved in perceptual constancy[7] Therefore, if a young bird must recognise its parent from a variety of angles it must have first seen its parent from all those angles This would in part be accomplished by rotations of the parent as it went about its business, but the process of attachment would be accelerated if, in addition, the young moved round the parent after familiarising themselves with one view Such a process of working for slight novelty balanced against a growing restriction of preference for what is familiar would not only make good biological sense, it would account for the birds preferring the unfamiliar object only in the early stages of imprinting[10]

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Microbial activation of prophenoloxidase from immune insect larvae

INTEREST in the infection of insects by parasites has been generated by the pathogenic potential of the parasite to the insect itself, or to a vertebrate host to which the parasite may be transmitted For example, because of the effects of malaria on man, the relationships between *Plasmodia* and various mosquito species have received much attention[1,2] An important part of the host-parasite relationship is the defence system of the host In insects, phenoloxidase (*o*-diphenol O_2 oxidoreductase, EC 1 10 3 1) has been suggested to be at least partially responsible for insect immunity and parasite resistance[3–5]

The enzyme produces quinones which can react with proteins[6] and with the thiol and amino groups of other compounds[6–8] Quinones can be bactericidal[9] When quinones are present in excess, the black pigment melanin is formed[6] A layer of melanin is frequently observed deposited on parasites by insects as part of their resistance The exact reactions of quinones with parasite tissues have not been determined, but by blocking quinone formation with phenylthiourea an insect's parasite resistance can be suppressed[5] Melanin deposition is often associated with insects' cellular response[5], but can also be a strictly humoral response[10] In some cases of unsuccessful malaria transmission by mosquitoes there is an apparent melanisation manifest in Ross black spores and degenerate oocysts[1,2] For quinones to be involved in resistance to an insect parasite, however, control of phenoloxidase activity would be essential Quinones randomly reacting with the insect host would be ineffective as antiparasitic agents Furthermore, large amounts of active phenoloxidase can kill insects[11] As a contribution to this problem, I describe here the activation of prophenoloxidase in the plasma of immune[12,13] *Galleria mellonella* larvae by microbial products

For immunisation, larvae (each larger than 200 mg) were injected with 1 0-μg doses of *Shigella flexneri* lipopolysaccharide B (Difco)[13] and incubated at 23° C Control larvae were uninjected Haemolymph was collected 18–24 h after immunisation and pooled in a CO_2 environment, at

Table 1 Enzyme activity in $(NH_4)_2SO_4$ fractions of immune plasma

	Protein	Phenoloxidase‡				Lysozyme
		Zymosan activation		α-Chymotrypsin activation		
$(NH)_4SO_4$ fraction*	mg g^{-1} lyophilisate†	U g^{-1} lyophilisate	Specific activity§	U g^{-1} lyophilisate	Specific activity§	U g^{-1} lyophilisate ‖
0–40% ppt , fraction *A*	10	0	0	43,950	4,395	0
40–50% ppt.	85	0	0	21,850	257	0
50–60% ppt	140	0	0	50	0	300
60% Supernantant, fraction *B*	66	0	0	0	0	14,300
Whole plasma¶	558	144,045	258	153,745	276	—

* Plasma was prepared from reconstituted haemolymph as described in the text Fractions were prepared by the addition of solid salt[18] (100% saturation = 70 6 g per 100 ml) Precipitates were dissolved in 0 01 M KPO_4^{-2}, *p*H 6 0 (the *p*H and ionic concentration—as determined by freezing point depression—of the plasma) Before testing enzymatic activities all fractions were dialysed against this buffer to remove the salt

† Assayed by the method of Lowry *et al* [19]

‡ 0 20 ml of a fraction was mixed with 0 10 ml of activator in water α chymotrypsin, 6 mg ml^{-1} zymosan, 1 mg ml^{-1} Mixtures were incubated for 5 min at 30° C, then assayed for phenoloxidase Values for water controls have been subtracted

§ U mg^{-1} protein

‖ ΔA_{450}, = -0 001 per min at 30° C of a suspension of *Micrococcus lysodeikticus* was one lysozyme unit[20]

¶ No salt added, no dialysis

least 20 larvae were used for each pool Care was taken to avoid gross contamination of haemolymph with fat body tissue To quantitate properly phenoloxidase activity per amount of haemolymph and to facilitate handling, pooled haemolymph was quick frozen with acetone-dry ice, lyophilised and stored at −20° C Haemolymph from control larvae was collected and stored in an identical manner

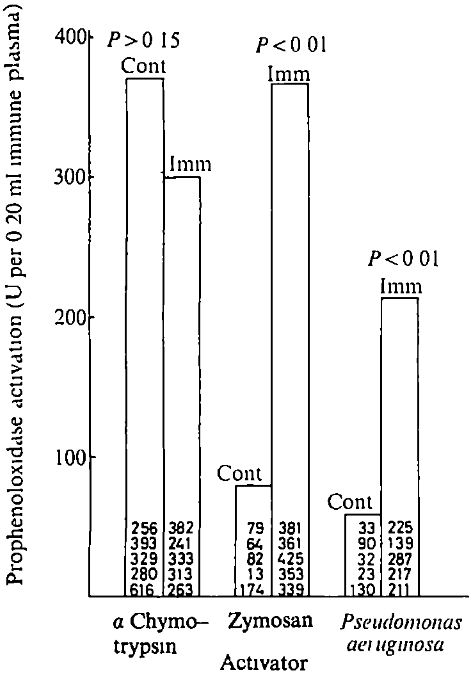

Fig. 1 Activation of prophenoloxidase in control (Cont) and immune (Imm) plasmas by various materials The values indicated by the bars are the average amounts of prophenoloxidase found in five different determinations A determination used 0 20 ml of plasma from a different pool of control or immune haemolymph mixed with 0 01 ml of activator solution or suspension, incubated for 5 min at 30° C, then assayed for phenoloxidase (See text for assay technique) Results for individual determinations are listed within each bar With no activator materials present—water controls—the phenoloxidase activities of plasmas ranged from zero to two units, these values have been subtracted Statistical comparisons are between control and immune plasmas α-Chymotrypsin (Nutritional Biochemicals Corp , three times crystalline) 6 mg ml^{-1} water Zymosan[16] (General Biochemicals) 1 mg ml^{-1} water *Pseudomonas aeruginosa* P11-1[12] was grown in nutrient broth +1% glucose and collected at 24 h by centrifugation, 8,000*g* for 15 min Cells were washed with 0 85% NaCl, then brought to $A_{530}=1$ 1, a concentration of about 5×10^9 bacteria per ml and frozen The cell suspension was thawed, resuspended in water and refrozen It was rethawed and heated at 100° C for 30 min After cooling, 0 10 ml aliquots were used to activate prophenoloxidase None of these activator materials had inherent phenoloxidase activity

My preliminary experiments and those of Evans[14] have shown that haemolymph phenoloxidase occurs as the proenzyme in plasma and can be activated by material from haemocytes To test for other types of activation, lyophilised haemolymph was reconstituted with distilled water to a concentration of 10 mg solids per ml This was 1/17 to 1/18 the usual concentration of solids in fresh haemolymph, and its purpose was to slow spontaneous activation of prophenoloxidase by haemocytes, so that plasma could be collected before activation The plasma and cellular fractions were separated by centrifuging whole reconstituted haemolymphs at 650*g* for 15 min at 4° C The supernatant fluids—the plasmas—were removed and used immediately When water was added to the haemocytes in the lyophilised material no lysis was observed microscopically Also, haemocyte concentrations were calculated, with correction factors for dilutions, to be 18,590 mm^{-3} for immune and 40,040 mm^{-3} for control reconstituted haemolymph, slightly higher than those reported by Stephens[15] for immune and control fresh haemolymph These observations tend to preclude massive haemolysis during handling although the release of some haemocyte materials into the plasmas was possible

Phenoloxidase activity was measured by spectrophotometric determinations of initial reaction rates with a Gilford recording spectrophotometer For a standard assay, 0 30 ml of test material was mixed with 4 7 ml of 0 1 M KPO_4^{2-}, *p*H 6 4, containing 20 μmol of 4-methylcatechol and 40 μmol of 4-hydroxyproline ethyl ester 4-Methylcatechol was enzymatically oxidised to its quinone The quinone reacted nonenzymatically with 4-hydroxyproline ethyl ester to form a stable product, 4-(4′-hydroxy-2′-carbethoxy-1′-pyrrolidyl)-5-methyl-*o*-benzoquinone, with a λ_{max} at 520 nm (ref 7) In the system used, with excess substrates, ΔA_{520} was directly proportional to enzymatic activity One phenoloxidase unit was defined as $\Delta A_{520}=+0$ 001 per min at 30° C, the maximum rate observed during the first 2 min after the initiation of the reaction was used By measuring the phenoloxidase activity of plasmas incubated with and without activator materials, prophenoloxidase activation was quantitated

Immune plasmas showed significantly more prophenoloxidase activation than control plasmas when both were incubated with either zymosan (a yeast polysaccharide)[16] or a preparation of damaged *Pseudomonas aeruginosa* (Fig 1) After treatment with α chymotrypsin (EC 3 4 4 5) both control and immune plasmas showed a high degree of prophenoloxidase activation, similar to that in immune plasmas treated with the microbial products

The activator(s) associated with zymosan and *P aeruginosa* did not have esterase activity towards benzoyl-L-tyrosine ethyl ester when tested at *p*H 6 0 (the *p*H of the plasmas) or at *p*H 7 8 The activator(s) in zymosan suspensions was associated not only with particles, but also with the particle-free solution It (they) passed through a 0 22-μm filter, but not through a dialysis membrane The zymosan activator(s) was stable at 100° C for 3 h, but after 3 h in 0 2 M H_2SO_4 at 100° C and then neutralisation with $Ba(OH)_2$ it was not found when assayed Activation associated with *P aeruginosa* was not manifest unless the cells were damaged by physical means or by pretreatment with egg white lysozyme (EC 3 2 1 17) (Fig 2) Egg white lysozyme does not lyse or kill *P aeruginosa*, but alters its cell wall[17]

Immune plasma was fractionated with $(NH_4)_2SO_4$ (Table 1) The material precipitated with 40% saturation (fraction *A*) contained most of the prophenoloxidase The material still in solution after 60% saturation (fraction *B*) had most of the lysozyme activity Prophenoloxidase in fraction *A* was activated by α chymotrypsin, but not by zymosan alone, however, the prophenoloxidase in fraction *A* could be activated by zymosan if fraction *B* was also added If egg white lysozyme was substituted for fraction *B*, activation did not occur The K_ms of phenoloxidase activated by zymosan and α chymotrypsin for 4-methylcatechol were similar, 1 20 and 1 07 mM respectively Figure 3 shows that when prophenoloxidase was diluted before activation by zymosan there was an exponential relationship between proenzyme concentration and activity, while dilution of the enzyme after activation gave an almost linear relationship

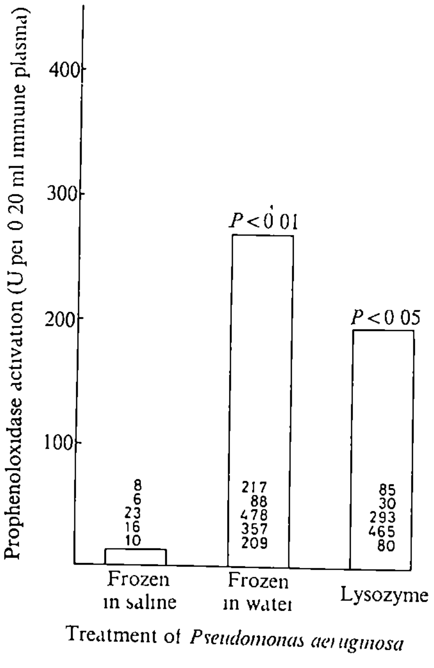

Fig 2 Manifestation of prophenoloxidase activator(s) in *Pseudomonas aeruginosa* after various treatments The activator(s) was determined by the activation of prophenoloxidase in immune plasmas The assay procedures were the same as for Fig 1 *P aeruginosa* cells frozen in 0 85% NaCl saline (see Fig 1), resuspended in water, but not refrozen or heated, served as controls Statistical comparisons are between these cells and those submitted to other treatments The cell suspension frozen in water was the *P aeruginosa* preparation used for Fig 1 Cells treated with lysozyme were frozen in saline, resuspended in 0 1 M KPO_4^{2-}, *p*H 7 0, with 10 mg lysozyme (Sigma, from egg white, three times crystallised) ml^{-1}, incubated 20 min at 30° C, and then resuspended in water Bacterial concentrations were the same for all three suspensions

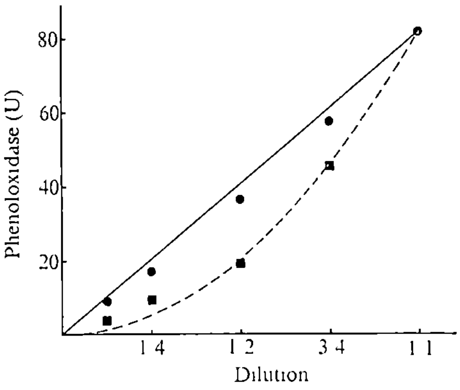

Fig 3 Effect of prophenoloxidase concentration in the zymosan activation mixture on the resulting phenoloxidase activity ■, Prophenoloxidase diluted before activation ($y=81x^2$) fraction *A* was brought to various dilutions with 0 01 M KPO_4^{2-}, *p*H 6 0 0 10 ml of a dilution was mixed with 0 10 ml of fraction *B*, and 0 10 ml of zymosan suspension, 1 mg ml^{-1} water Each of these mixtures was incubated for 5 min at 30° C, then assayed for phenoloxidase ●, Phenoloxidase diluted after activation ($y=81x$) fraction *A*, fraction *B*, and zymosan suspension were mixed 1 1 1 (v v v), then incubated at 30° C After 5 min, various dilutions of this mixture were made using a mixture of 0 01 M KPO_4^{2-}, *p*H 6 0, and zymosan suspension, 2 1 (v v) Immediately after all dilutions had been prepared, 0 30 ml aliquots were assayed for phenoloxidase activity These assays were completed within 15 min

Activation of prophenoloxidases is associated with the aggregation of enzyme subunits[18,21] An aggregation mechanism is consistent with the concentration effect shown in Fig 3 Haemolymph lysozyme in fraction *B* may partially digest zymosan material which then acts as a site for the adsorption, aggregation and activation of prophenoloxidase subunits This would explain the differences observed between control and immune plasmas in Fig 1, since immune haemolymph contains larger concentrations of lysozyme[22] Another hypothesis is that zymosan activates a proteolytic enzyme in fraction *B*, as it does in complement[23] The proteolytic enzyme would in turn activate the prophenoloxidase

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Elevation of total serum IgE in rats following helminth parasite infection

HELMINTH parasites provide the most potent stimulus known for the biosynthesis of IgE In every species which has been studied, infections with parasitic worms lead to the production of high levels of reaginic or IgE antibodies against parasite antigens[1,2] and, in man, greatly elevated levels of total IgE occur in most individuals infected with any one of a variety of helminths including *Ascaris lumbricoides*[3], *Capillaria philipinensis*[4] and ancylostomes[5]

Experiments in the rat have indicated how the IgE stimulating effect of helminth parasites may operate In this species, infections with *Nippostrongylus brasiliensis* or *Fasciola hepatica,* as well as stimulating parasite specific IgE antibodies, cause nonspecific potentiation of unrelated IgE responses to such antigens as egg albumen, keyhole limpet haemocyanin, and house dust[6–8] Present evidence indicates that live worms produce a factor which, acting via T cells[9], has the capacity to stimulate those B cells which have been programmed for IgE production by previous immunisation In so far as IgE responses against different antigens may be simultaneously potentiated[8] it seems likely that all the existing IgE responses of the animal are affected

Here we now present evidence which supports this We have compared the level of total serum IgE in rats following conventional immunisation with that of rats whose response has been potentiated by subsequent infection with helminth parasites In the latter, the amount of total serum IgE is much greater, and far more than can be ascribed to IgE antibodies specific for the immunising antigen or the parasite

Until recently it has not been possible to estimate total serum IgE in the rat, or indeed in any other species but man, for lack of specific antiserum to this immunoglobulin The recent discovery of a strain of rats bearing IgE immunocytomas[10, 11] has overcome this difficulty since they provide a source of myeloma protein from which purified IgE can be isolated in relatively large amounts Such a purified IgE preparation has been used to induce specific antiserum to rat IgE and also as a reference preparation in an assay for rat total serum IgE using the radioactive single-radial-diffusion technique of Rowe[12]

The specific immunological reagents were prepared as follows The purification of rat IgE from the serum or rats bearing IgE secreting immunocytomas was as previously described[10] with the following modifications Ammonium sulphate precipitation was performed at 50% final concentration, and as a supplementary step preparative agarose electrophoresis was done in 1% agarose equilibriated with 63 mM sodium barbiturate, 15 mM Barbital, 1 8 mM calcium lactate buffer, adjusted to pH 8 6 with HCl The electrophoresis was carried out for 1 h at 20 V cm^{-1} After electrophoresis, the position of the monoclonal IgE was located by comparison with a fixed and stained agarose gel electrophoretic pattern of the same IgE preparation The agarose-containing myeloma IgE was cut out and removed from the slide and the proteins were extracted from the gel by pressure Goat antiserum to rat IgE was obtained by immunisation with purified antigen (IR2 monoclonal protein) emulsified in complete Freunds adjuvant Antisera were absorbed with a solid immunoabsorbant[13] made of serum from germ-free rats Antiserum to goat IgG was prepared by immunisation of rabbits with goat IgG (prepared by the caprylic acid method)[14] in complete Freunds adjuvant The antiserum was also purified by the caprylic acid technique and the resultant IgG fraction was labelled with ^{125}I by the method of McConahey and Dixon[15]

Dilutions of anti-rat IgE in the agar from 1 100 to 1·4,000 could be used The last dilution gave the greatest sensitivity enabling estimation in the range of 0 35-22 24 μg IgE ml^{-1} The reference preparation (IR162 Temoin) contained 5 7 mg ml^{-1} of rat IgE

Outbred female Hooded Lister rats weighing 150–200 g were used in the experiments described here Twenty normal rats (Group 1) were bled before treatment and were then

Table 1 Reaginic antibody titre and total serum IgE (mean ± s e) in serum from normal, immunised infected and potentiated rats

Group	Treatment of rats	Passive cutaneous anaphylaxis* titre		Total serum IgE (μg ml^{-1})
		Egg albumen	*N brasiliensis*	
1	Normal rats no treatment	—	—	most rats <0 35
2	Primary response to 1 μg EA†	92 ± 36	—	1 33 ± 0 25
3	Secondary response to 10 ng EA	1,228 ± 231	—	1 80 ± 0 39
4a	Potentiated reagin response to EA 12 d after *N brasiliensis*‡	1,316 ± 270	—	288 ± 47
4b	Not potentiated reagin response to EA 12 d after *N brasiliensis* (pool of 12 sera)	4,096	—	493
5	Unimmunised rats 12 d after *N brasiliensis* infection	—	—	247 ± 42
6	Unimmunised rats 18 d after *N brasiliensis* infection	—	640 ± 109	104 ± 8

*To estimate specific reaginic antibodies by the passive cutaneous anaphylaxis technique[17], 0 1 ml quantities of saline dilutions of the test sera were injected intradermally in Hooded Lister recipient rats each injection being duplicated on a different animal 48–72 h later, the animals were injected intravenously with 2 5 mg of EA or 0 5 ml *N brasiliensis* antigen (saline extract of 500 homogenised worms) together with 0 5 ml of 1% Evans blue which acts as an indicator of vasodilation The skin reactions were examined after 20 min and the titres recorded above are the greatest dilutions of serum which gave reactions larger than 5 mm in diameter

†Egg albumen

‡The method of culture of *N brasiliensis* and preparation of the infective dose have been previously described[18,19]

immunised with 1 μg egg albumen (Sigma grade V) injected intraperitoneally together with 10^{10} *B pertussis* organisms (Wellcome Biological Reagents) They were bled again 20 d after immunisation (Group 2) and were then divided into two groups and treated as follows Group 3 rats received a booster injection of 1 μg egg albumen and were bled 4 d later, while Group 4a rats were infected by subcutaneous inoculation of 4,000 *N brasiliensis* larvae and were bled 12 d later These times of bleeding were determined by previous observations of the timing of peak secondary and potentiated reagin responses respectively[6,16]

Table 1 shows the specific reaginic antibody levels and total serum IgE in the serum of these animals It is evident that the second injection of egg albumen although resulting in a good secondary antigen-specific IgE response (Group 3) did not have a marked effect on total serum IgE Infection with *N brasiliensis* on the other hand (Group 4a), as well as causing a similar elevation of egg-albumen specific IgE resulted in a most dramatic elevation of total serum IgE from 1 33–288 μg ml^{-1} This level is well in excess of that which, by comparison of the egg-albumen reagin titres of the two groups, can be accounted for by egg-albumen reagins alone Equally the parasite-specific response cannot be implicated at this time after infection since anti-*N brasiliensis* reagins do not normally appear until later in the infection and no parasite specific reagins could be detected in these areas A pool of serum from another group of rats with a very marked potentiated reagin response to egg albumen (Group 4b) showed even greater levels of total serum IgE

The remaining two groups shown in Table 1 were 10 normal animals infected with *N brasiliensis* without previous immunisation Group 5 were bled 12 d after infection with 4,000 larvae at which time no parasite specific reagins were present Greatly elevated levels of total serum IgE occurred in this group in the absence of IgE antibody responses to any known antigens Group 6 were bled 18 d after infection when parasite specific IgE was present, but when the level of total serum IgE had actually declined

The results following infection with the parasite *Fasciola hepatica* have been essentially similar to those we report here

We have also estimated total serum IgE in Wistar rats and find that animals of this strain do not produce levels as high as those of the Hooded Lister strain For instance, 12 d after *N brasiliensis* infection the IgE level in Wistar serum (five rats) was 25 3±5 6 μg ml^{-1} It has been reported that Hooded Lister rats have an exceptional ability to produce IgE antibodies, in that prolonged responses are induced by immunisation with small doses of conventional antigen and that marked secondary responses can occur[16] It is interesting that this facility is also reflected in very high levels of total serum IgE (up to 493 μg ml^{-1}) following helminth infection in rats of this strain

The main points which emerge from these results are that infection of the rat with helminth parasites leads to a great elevation of total serum IgE and that the bulk of this IgE is surplus to levels of IgE antibody against known antigens It would seem reasonable to assume that this IgE consists at least in part of a number of potentiated reagin responses to miscellaneous unknown antigens to which the animal has previously become sensitised By analogy it is of course tempting to suppose that the high levels of total serum IgE seen in parasite infected human beings consist not exclusively of antiparasite IgE responses to unrelated antigens

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Alternative route for nitrogen assimilation in higher plants

IT is generally considered that glutamate dehydrogenase (EC 1 4 1 3) is the enzyme that is chiefly responsible for the incorporation of nitrogen into the α-amino group of amino acids

$$\alpha\text{-oxoglutarate}+NH_4+NAD(P)H+H^+$$
$$=\text{L-glutamate}+H_2O+NAD(P)^+ \qquad (1)$$

We suggest that this is not the case in the leaves of higher plants

There is considerable evidence that chloroplasts are a major site of α-amino nitrogen production[1–5] but, although the chloroplast contains glutamate dehydrogenase, the level of activity is low and the enzyme has a high K_m for ammonia[5], greater in fact than the concentration of ammonia required to uncouple chloroplasts Chloroplasts also contain glutamine synthetase which has a much lower K_m for ammonia and a much higher activity[4 6,7] If glutamine synthetase is the route of entry for ammonia, how-

Table 1 Glutamate formation by intact chloroplasts in the presence of various amino donors in the light

Nitrogen donor	Acceptor	Glutamate formed (μmol per mg chlorophyll h^{-1})	Glutamine lost (μmol per mg chlorophyll h^{-1})
None	α-oxoglutarate	0 4	—
Glutamine	α-oxoglutarate	6 9	3 6
NH_4Cl	α-oxoglutarate	0 5	—
Asparagine	α-oxoglutarate	3 5	—
Aspartate	α-oxoglutarate	4 8	—
Glutamine	None	0 7	0

The reaction mixture consisted of intact chloroplasts containing about 100 μg chlorophyll, 0 5 μmol α-oxoglutarate and 0 5 μmol of amino donor except asparagine which was 1 25 μmol, in a final volume of 0 3 ml of 0 33 M Sorbitol, 5 mM potassium chloride and 10 mM sodium pyrophosphate, *p*H 7 5

ever, some way must exist to transfer the amide group of glutamine to the amino group of glutamate Such an enzyme, glutamate synthase (EC 2 6 1 53), also known as GOGAT, L-glutamine α-oxoglutarate aminotransferase (NADPH-oxidising) (equation (2)), has been shown to be present in bacteria[8] and yeasts[9], particularly when grown under limiting concentrations of ammonia, and in nitrogen-fixing bacteria and the bacteroids of nodulated higher plants[10,11]

$$\text{L-glutamate} + \alpha\text{-oxoglutarate} + \text{NADPH} + \text{H}^+ = 2\ \text{L-glutamate} + \text{NADP}^+ \qquad (2)$$

This pyridine nucleotide-linked enzyme has not been reported in the tissues of higher plants in spite of attempts by ourselves and others[7,11] to find it Here we present evidence to show that chloroplasts can perform a light-dependent synthesis of glutamate from α-oxoglutarate and glutamine and that extracts of chloroplasts contain a ferredoxin-dependent glutamate synthase

Chloroplasts from 10–15-d-old *Pisum sativum* (var Meteor) plants were extracted by a method based on that of Walker[12] in a medium containing 0 33 M sorbitol, 1 mM $MgCl_2$, 2 mM EDTA, 1 mM mercaptoethanol, 10 mM sodium pyrophosphate, *p*H 7 0 The chloroplast pellet was washed in 0 33 M Sorbitol, 5 mM KCl, 10 mM sodium pyrophosphate, *p*H 7 5 and then resuspended in the same medium Aliquots containing 100–150 *μ*g chlorophyll were incubated with substrates for 20 min at 30° C The reaction was stopped by the addition of ethanol Amino acids were separated by paper chromatography in 75% (w/w) phenol, in the presence of ammonia vapour, and quantified by the method of Atfield and Morris[13]

The results (Table 1) show that chloroplasts are capable of converting α-oxoglutarate to glutamate in the light, confirming the findings of earlier workers[14] They also confirm that this reaction is not stimulated by ammonia—the substrate for glutamate dehydrogenase When glutamine is supplied, however, there is a seventeenfold stimulation in the rate of glutamate formation and the reaction is absolutely light dependent The only reaction shown in Table 1 that occurred in the dark was between aspartate and α-oxoglutarate, which was at the same rate as determined in the light Asparagine also markedly stimulates the conversion of α-oxoglutarate to glutamine The light dependence of the reaction, compared with the light independence of transaminase activity, and the inability of ammonia to act as an amino group donor would suggest that asparagine is not being cleaved by asparaginase to give aspartate and ammonia which could be subsequently incorporated

The results with glutamine are best explained by the presence of glutamate synthase To test this, chloroplasts prepared and washed as described above were resuspended in 0 1 M tricine, *p*H 7 5 After standing for 30 min the ruptured chloroplast debris was centrifuged off and the supernatant passed over a Sephadex G-75 column pre-equilibrated with 0 1 M tricine pH 7 5 The chloroplast extract was eluted with the same buffer Aliquots of the extract were incubated with various substrates for 20 min at 30° C The reaction was stopped and the amino acids separated and determined as described above

Preliminary studies with a spectrophotometric assay confirmed that these extracts had no NADPH dependent glutamate synthase and only a trace of glutamate dehydrogenase activity But experiments using methyl viologen and dithionite as an electron donating system showed the presence of a glutamine and α-oxoglutarate dependent synthesis of glutamate (Table 2) Subsequent studies showed that ferredoxin is an extremely effective donor, none of the other donors tried was capable of supporting the reaction The stoichiometry of the reaction is two molecules of glutamate formed for each glutamine utilised, as predicted by equation (2) The rates of glutamate synthesis expressed on a chlorophyll basis are of the same order of magnitude as those in the intact chloroplasts and are also similar to those of nitrite reduction and α-amino nitrogen production measured in other studies[2,4]

The demonstration of a ferredoxin-dependent glutamate synthase in the chloroplasts completes a chain of reactions for the incorporation of nitrate into α-amino nitrogen (Fig 1) Several considerations suggest this is the major

Table 2 Glutamate formation by chloroplast extracts under various conditions

Electron donor	Nitrogen donor	Acceptor	Glutamate formed (μmol per mg chlorophyll h^{-1})	Glutamine lost (μmol per mg chlorophyll h^{-1})
Experiment 1				
Fd + DIT	None	α-oxoglutarate	0*	—
Fd + DIT	Glutamine	α-oxoglutarate	23 2	10 8
Fd + DIT	NH_4Cl	α-oxoglutarate	0	—
Fd + DIT	Asparagine	α-oxoglutarate	0	—
Fd + DIT	Aspartate	α-oxoglutarate	2 08	—
None	Aspartate	α-oxoglutarate	1 55	—
Fd + DIT	Glutamine	None	0	0
Experiment 2				
None	Glutamine	α-oxoglutarate	0	0
Fd + DIT	Glutamine	α-oxoglutarate	9 6	4 9
MV + DIT	Glutamine	α-oxoglutarate	2 8	1 3
NADPH	Glutamine	α-oxoglutarate	0	0
NADH	Glutamine	α-oxoglutarate	0	0
FMN + DIT	Glutamine	α-oxoglutarate	0	0
Fd	Glutamine	α-oxoglutarate	0 5	0 1
DIT	Glutamine	α-oxoglutarate	0 5	0 2

The reaction mixture consisted of 70 μmol tricine, *p*H 7 5, chloroplast extract equivalent to approximately 100 μg chlorophyll, 3 5 μmol α-oxoglutarate and the electron donors and nitrogen donors as indicated The final volume of the reaction mixture was 0 7 ml The amounts of electron donors in the reaction mixture was as follows ferredoxin (Fd) 0 1 mg, sodium dithionite + $NaHCO_3$ (DIT) 1 6 mg each, FMN 0 2 mg, methyl viologen (MV) 0 1 mg, NAD(P)H 0 4 mg Amino donors were present at 2 mM concentration except asparagine which was at 5 mM Ferredoxin was derived from *Spirulina maxima*

*No visible glutamate spot on the chromatogram

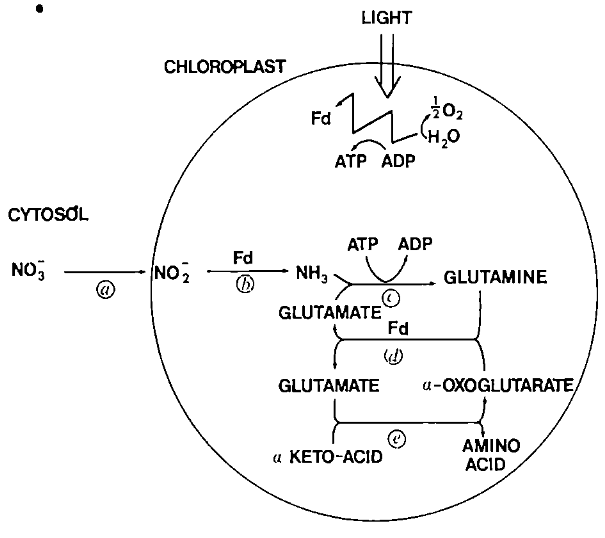

Fig 1 The proposed route of entry of nitrate into amino acids in leaves The enzymes are, *a*, nitrate reductase, *b*, nitrite reductase, *c*, glutamine synthetase, *d*, glutamate synthase and *e*, transaminase

route of entry of nitrogen into the organic form in the leaves of higher plants The leaf normally receives its inorganic nitrogen in the form of nitrate This nitrate is metabolised through nitrate and nitrite reductases to ammonia Both of these reactions are strongly light stimulated[2,3] as is the incorporation of $^{15}NO_3$, $^{15}NO_2$, and $^{15}NH_3$ into α-amino nitrogen[15] Nitrite reductase is located in the chloroplasts and the presence of glutamine synthetase with a low K_m for ammonia ensures that the ammonia formed can be incorporated without building up to levels which would uncouple the chloroplasts The presence of glutamate synthase does not preclude a role for glutamate dehydrogenase in ammonia assimilation, particularly under conditions of high ammonia availability These are most likely to occur in roots Since ammonia is not normally a major nitrogen constituent of the xylem sap[16] it is unlikely that it occurs in any concentration in the leaves unless it is produced by deamination of transported amines and amino acids

The operation of the route in Fig 1 would result in the same pattern of incorporation of the nitrogen of NO_3, glutamate and the amide group of glutamine into all the amino acids The results of Lewis and Pate[17] with ^{15}N feeding studies confirm that this occurs in leaves This is distinct from the traditional idea of incorporation of glutamine into relatively few amino acids, as suggested for yeast by Sims and Folkes[18] The ^{15}N studies of Baker and Thompson[19] with *Chlorella* are also consistent with Fig 1

The results we present suggest that asparagine is metabolised by chloroplasts although not by the same enzyme as glutamine It is probable that the amide is transferred to another acceptor molecule (such as, pyruvate, phosphoenol pyruvate or oxoaloacetate) in the presence of a reductant or to glutamate to form glutamine and pass into the chain The strong light dependence of glutamate synthesis from asparagine is consistent with the rapid disappearance of asparagine from leaves in the light

We suggest that the leaves of higher plants contain a glutamate synthase which plays a crucial role in nitrogen assimilation This enzyme differs from those previously described in being ferredoxin dependent Its presence helps to explain a major anomaly of intermediary nitrogen metabolism in higher plants, namely the unsuitability of glutamate dehydrogenase as a major point of entry of nitrogen into organic combinations in chloroplasts

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Note added in proof Since this paper was submitted we have become aware of a report[20] on NAD(P)H-dependent glutamate synthase type activity in extracts of carrot cell cultures

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Evolution of cell senescence, atherosclerosis and benign tumours

IT has been proposed that cell senescence is the cause of organismic senescence[1–3] and that senescence is under evolutionary control[4] Orgel[5] has argued that cell senescence could not be under evolutionary control because it is difficult to imagine how senescence of the organism has a positive evolutionary advantage

I propose that cell senescence itself, rather than senescence of the organism, is the genetically controlled and programmed event selected by evolution and that any effects on somatic senescence is a pleiotropic effect of this function The advantage of programmed cell senescence to the organism is that it stops cells which have escaped from normal control from dividing indefinitely If a cell with potential for further division gets out of place in the body, it is possible for it to settle in a new location where the normal environmental signal inhibiting division is lacking Were these now uninhibited cells capable of unlimited growth, they would divide to fill the entire space available A limited number of cell divisions, however, would limit the size of the growth, thus saving the organism

In vitro normal human fibroblasts are limited in the number of divisions[1,2]. The life of a culture of cells from a tissue explant can be divided into three phases[1]. Phase I ends with the formation of the first monolayer; phase II refers to a period when rapid growth occurs in proper culture conditions, while phase III refers to the period of senescence of the culture. Not only is the percentage of non-dividing cells higher in phase III cells than phase II cells[6], but the growth rate of dividing cells is is reduced[7]: so all cells of the culture are affected by senescence. Phase II cells, however, are unaffected by being mixed with phase III cells[2].

Phase II cells can be held for long periods in a non-dividing state without affecting the number of divisions the cells undergo before senescence[8]. Thus, the metabolic age of a culture can be separated from the doubling age. The onset of senescence is shown to be dependent on the doubling age and the doubling age of a culture can be estimated[9].

Growth of a clone which arose from a single cell is restricted to the edge. The cells in the centre are inhibited by contact inhibition. Thus, the cells in the centre are at a younger divisional age than the cells near the edge[10]. As the cells go out from the centre, they become progressively older and growth stops at the outer edge of the colony when the cells there have divided the maximum number of times and senesce.

The evidence also indicates that cells senesce *in vivo*. With increasing age of the human donor there is a reduction in the number of cell doublings before senescence in culture[11]. Serial transplants of normal tissue into young inbred animals die out[12–14] and the death of the tissue is related to number of cell divisions rather than to its metabolic age[15]. In contrast, transformed cells (malignant cells) which do not show contact inhibition and do not senesce in tissue culture, can be transferred *in vivo* indefinitely[12].

Hayflick postulated that organisms age because their cells age[2,3] and this relationship has been supported by studies on Werner's syndrome, a genetic disease characterised by the development in early adulthood of a wide variety of degenerative features similar to those found in old age. Cells from sufferers of this syndrome are already senescent, doubling less than ten times in tissue culture[11], while cells of normal people of similar age double 35 times. Cell senescence is not necessarily the only cause of organismic senescence since humans aged 70–90 yr are senescent; but fibroblast cultures from them are not senescent, doubling at least twenty times in tissue culture[11], which is over 40% of the doublings found in human embryo cultures.

Atherosclerotic lesions consist of 'plaques' of fibroblasts lining the interior of the great arteries. Limitation in the size of these plaques provides a strong selection for cell senescence.

Benditt and Benditt[16] present the following evidence that a single plaque is monoclonal; that is, all of the cells of a plaque are derived from a single dividing precursor cell. They looked at plaques from the three females who were heterozygous for two electrophoretic variants of the X-linked glucose-6-phosphate dehydrogenase. There is random inactivation of one or the other of the X chromosomes at about the 1,000 cell stage of embryo development, consequently each heterozygous female has a mixture of cells with one or the other X chromosome inactivated[17]. When small pieces (0.1 mm^3) were taken from the aorta wall, both enzyme types were present. But when larger pieces from plaques (0.5 mm^3 to 20–30 mm^3) were taken, most of them had only one isozyme present. It is unlikely that these plaques are scars[18] formed where the artery had been injured, since hypertrophic scar tissue is polyclonal[16].

Cells escaping into the blood stream from points where there is injury to the vascular system would be carried away from the wound by blood flow and could settle on the inner wall of arteries. Possibly they have trouble settling or dividing except where there are fatty streaks, thus giving the correlation between fats and atherosclerosis. The fibroblasts, now living in a new place in the body, exposed to a high velocity flow of nutrient rich blood, would no longer be contact inhibited[19] and would start dividing. As the single cell forms a clone, the cells in the centre away from the flow of blood, will cease dividing, while the cells on the edge will continue to divide. If these cells were able to continue dividing they would form quite a large growth and constrict the artery. Since they are limited to a certain number of divisions, however, the size of the plaque is limited in the same way as the size of a colony arising from a single cell in tissue culture[10].

I am postulating that atherosclerotic plaques arise from cells which escape into the arteries, settle and start growing. To keep these plaques at a minimum size, evolution has selected for a 'clock' which limits the number of cell divisions. This number is set by the conflicting demands of the large number of divisions required for wound repair and cell replacement and the restriction in the number of cell divisions needed to control atherosclerotic plaques and other abnormal growths.

The theory does make several predictions which can be tested. The first is that plaques will grow quickly and then stop when the cells on the outer surface senesce. Second, with fully grown but relatively young plaques, the cells at the centre of the plaque should be able to divide in tissue culture and the ones at the edge should not. Third, the number of divisions the cells from the centre of a plaque can go through should be correlated with the size of the plaque, given that the plaque started from a single cell; and last, the median final size of a plaque should be correlated with the age of the person at the time the cells were released into the blood stream.

Limitation in the size of some benign tumours could also provide strong selection for cell senescence. It has been shown that leiomyomas are monoclonal[20]. Many leiomyomas seem to originate near arteries[21] and thus may have arisen from cells from arterial walls which have escaped into the lyometrium of the uterus. Similar predictions as those made for atherosclerotic plaques apply to such benign tumours. Other benign tumours particularly the large ones, could originate from other causes, such as by a mutation which destroys the system providing contact inhibition. In this case, all the cells, not just the ones on the edge, would go through the maximum number of divisions.

So far no mechanism has been proposed as to how cell senescence takes place. The theory presented above explains how natural selection can select for cell senescence and implies that cell senescence is under genetic control. Views on cell senescence may be divided into two groups: one favours an evolutionary-genetic basis, as in this paper, and the other believes that senescence is caused by random events which create disturbances within the cell which are not fully correctable and that this accumulation of errors eventually leads to death. Any random error theory, such as the 'error catastrophe theory'[15], must be able to explain why cultures of transformed cells are not severely enough affected by the errors to senesce and why normal cells can not evolve the state of perfection enjoyed by transformed cells. Moreover, any random error theory needs to explain why germ line cells are less affected by the errors than the somatic cells. The simplest explanation to these problems is that senescence is under genetic control and that induction of senescence takes place during differentiation leaving the germ line unaffected.

Any mechanism, such as that proposed by the 'error catastrophe theory', could be used by evolution as the means to induce senescence. But a special compound could be produced whose sole function is to induce senescence.

Milo[22] has presented evidence that a glycoprotein extracted from the cell wall of senescent phase III cells causes almost immediate senescence when put on phase II cells.

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Insulin stimulates myogenesis in a rat myoblast line

THE clonal rat myoblast cell line L_6 isolated by Yaffe[1] undergoes myogenesis when the mononucleated myoblasts become confluent and fuse into multinucleated myotubes. This morphological differentiation is correlated with the synthesis of characteristic muscle proteins such as myosin and creatine kinase[2]. When L_6 myoblasts fuse the specific activity of adenylate cyclase[3] decreases and cyclic AMP, dibutyryl cyclic AMP and theophylline reversibly inhibit the differentiation process[4]. In primary cultures of rat muscle cells, a decrease in cyclic AMP has been reported at the time of fusion[5]. These facts support the hypothesis that low levels of cyclic AMP correlate with myoblast differentiation, while high levels inhibit fusion. In view of the relationship between the action of insulin and the biosynthesis of cyclic nucleotides[6] and the effects of the hormone on muscle metabolism[7], we have examined the influence of insulin on myogenesis of cultured L_6 myoblasts. The hormone replaces partially the serum requirement for morphological differentiation of primary cultures of chick embryo myoblasts[8,9], and now we have found that it stimulates fusion and the specific activity of creatine kinase in L_6 myoblasts in the presence or absence of serum.

In an attempt to separate the effects of insulin on myogenesis from those on metabolism, we examined the effect of the hormone on myoblast fusion in the presence of different concentrations of serum (Fig. 1). In serum-free medium almost no myotubes were observed in the absence of insulin and many cells died (Fig. 1*a*). In 1% serum, myotubes formed after 2 d and in 10% serum they appeared after 4 d (Fig. 1*c* and *e*). Addition of insulin (1 μg ml^{-1}) to the medium stimulated both the rate and extent of fusion at all serum concentrations tested (Fig. 1*b*, *d* and *f*), most of the nuclei being incorporated into myotubes within 24 h of the onset of fusion. Myotubes formed in the presence of insulin were much larger than those formed in its absence,

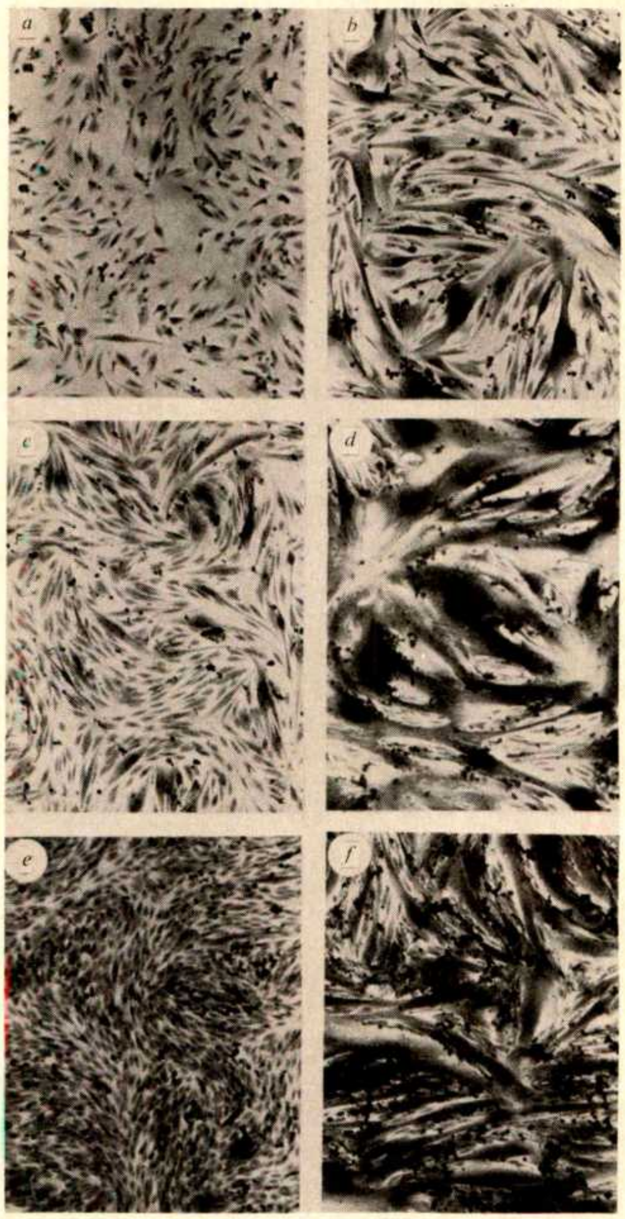

Fig. 1 Effect of insulin and serum on myoblast fusion. Rat myoblast L_6 cells (from Dr S. Heinemann, Salk Institute) were grown at 37° C in a humidified incubator under a 5% CO_2 95% air atmosphere in complete medium (Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum, Grand Island Biological Co.). To study fusion, cells were plated in complete medium at 8×10^3 cell cm^{-2} in 35 mm dishes. This medium was removed 24 h later, the dishes were rinsed with phosphate-buffered saline, and re-incubated under the following conditions. *a* and *b*, Serum-free medium containing 1.5 mg ml^{-1} bovine serum albumin (BSA); *c* and *d*, medium containing 1% foetal calf serum; *e* and *f*, complete medium (10% foetal calf serum). Insulin (1 μg ml^{-1}, zinc-free, from Dr C. Yip, University of Toronto) was added when the medium was changed for *b*, *d*, and *f* and the dishes were stained with methylene blue 3 d later.

with nuclei located more centrally. They also tended to degenerate more rapidly[8].

We then found that in the presence of insulin the specific activity of creatine kinase increased markedly at three serum concentrations (Fig. 2). The increase coincided with the onset of fusion (data not shown). The highest specific activity was reached in cultures grown in 1% serum in the presence of insulin, and under these conditions the maximum was reached in only 48 h (Fig. 2*b*). The increase in creatine kinase activity (as well as in morphological fusion) was delayed by about 24 h in cultures containing 10% serum (Fig. 2*c*). By changing the insulin-containing medium at 4 d, we obtained a further stimulation of creatine kinase levels in the presence of 10% serum, but not 1% serum (dashed lines, Fig. 2*b* and *c*). In other experiments the glycogen phosphorylase content of myotubes, detected histochemically[2], also increased after addition of insulin. It is interesting that de la Haba *et al.* found no accumulation of the characteristic muscle enzyme glycogen synthetase in primary cultures of myoblasts fused in the presence of insulin in serum-free medium[9]. Since under similar conditions with the L_6 myoblast line we observed a marked stimulation of creatine kinase activity by insulin, either there are differences in the response to insulin of primary cultures and L_6 cells, or glycogen synthetase and creatine kinase levels are controlled independently during myogenesis.

All the above experiments were carried out using 1 μg ml^{-1} of insulin. When we used 10^{-2} μg ml^{-1} insulin (Fig. 3*a*) we observed a significant stimulation of morphological fusion and creatine kinase activity: this concentration is about one order of magnitude higher than that found *in vivo*. When the concentration of serum was low, the maximum stimulation was obtained with 1 μg ml^{-1} insulin, while in 10% serum no plateau was apparent even with 5 μg ml^{-1} insulin. The requirement for high concentrations of insulin for maximum stimulation of creatine kinase may be due in part to inactivation of the insulin, especially in 10% serum (compare Fig. 2*c*). It may also indicate that a continuous and prolonged exposure to insulin is needed for the increase in fusion and creatine kinase activity. This possibility was tested by exposing cultures to insulin for various times, up to 4 d, removing the hormone, then measuring creatine kinase activity at 4 d. Figure 3*b* shows that insulin had to be present for more than 24 h to stimulate significantly creatine kinase specific activity, and longer exposure resulted in greater enzyme activity.

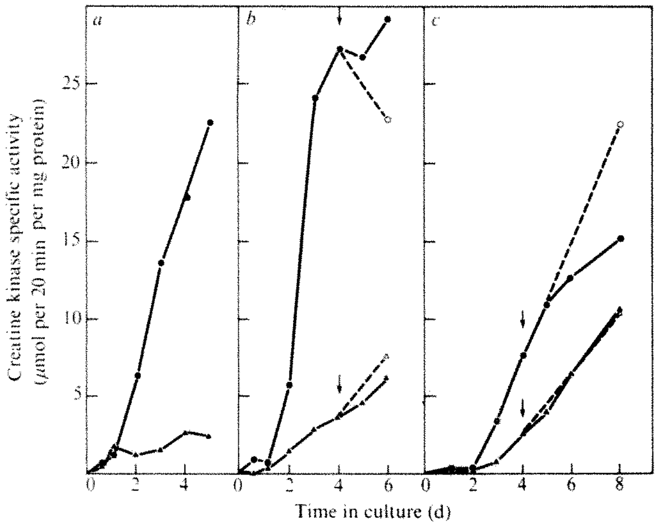

Fig. 2 Effect of insulin and serum on specific activity of creatine kinase. Cells were plated as described in the legend to Fig. 1, and the medium was replaced by medium containing various concentrations of serum in the presence or absence of insulin (1 μg ml^{-1}). At the indicated times after the medium change, the medium was removed, the cells were rinsed quickly with buffer *A* (100 mM Tris-acetate, *p*H 6.8 (22° C), 25 mM magnesium acetate, 10%, v/v, glycerol), drained, and kept frozen at −35° C. After thawing, cells were scraped into 0.35 ml buffer *A* supplemented with 0.2%, v/v, Nonidet P-40, and 0.1 mM phenymethylsulphonylfluoride (Sigma) and subjected to two cycles of freezing and thawing. Aliquots from duplicate plates were assayed for creatine kinase[10] and protein[11]. Variations in specific activity were usually less than 15% for values greater than 1 μmol per 20 min per mg protein. *a*, Serum-free medium containing 1.5 mg ml^{-1} BSA; *b*, medium containing 1% foetal calf serum; *c*, complete medium (10% foetal calf serum). ▲, Minus insulin; ●, 1 μg ml^{-1} insulin. The open symbols indicate values obtained after replacing the medium on day 4 (arrows) with fresh medium with (○), or without insulin (△).

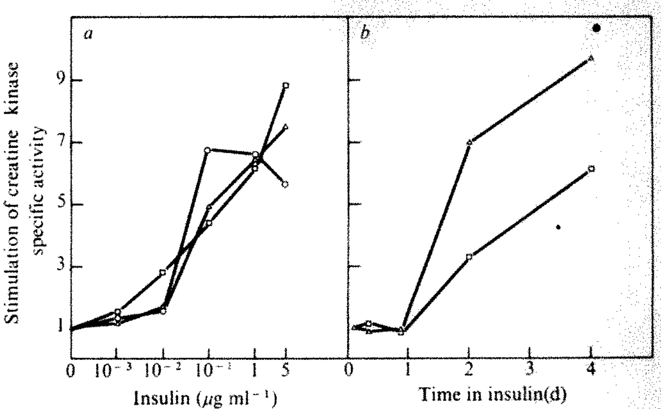

Fig. 3 Characteristics of the stimulation of creatine kinase by insulin. Cells were handled as described in the legend to Fig. 1, insulin was added at the time of the medium change, and cells were frozen 4 d later for determination of specific activities as described in the legend to Fig. 2. Results are expressed as relative stimulation over the control incubated without insulin. *a*, Insulin concentration dependence. Control specific activities were 1.0, 1.0 and 0.6 μmol per 20 min per mg protein in 0, 1% and 10% serum respectively. *b*, Dependence on time of exposure to insulin. Insulin (1 μg ml^{-1}) was added at time 0 for various times after which the medium was removed and replaced by medium at the same serum concentrations but lacking insulin. Initial specific activities without insulin were 1.3 and 0.6 μmol per 20 min per mg protein in 1% and 10% serum respectively. ○, No serum; △, 1% serum; □, 10% serum.

Insulin stimulates many cellular activities, especially in muscle cells[7], and its observed effect on myogenesis in both L_6 cells and primary cultures[8] may not be a specific effect on differentiation, but an indirect result of a general stimulation of cellular metabolism, especially in serum-free medium. For example, in fibroblasts serum starvation results in a reduction in RNA and protein synthesis, and these activities can be restored by the addition of insulin[12]. In the case of L_6 myoblasts, however, cell density and protein synthesis are higher in 10% serum minus insulin than in serum-free medium plus insulin, yet myogenesis is increased in the latter case. (We have also found that insulin does not increase RNA or protein synthesis in the first 30 h after its addition to cells grown in 10% serum; a twofold stimulation was obtained later at 48 h during an 8 h label period.) Thus, although further experiments are needed, we believe it unlikely that the stimulation of the specific activity of creatine kinase and the morphological changes caused by insulin are due to nonspecific stimulation of protein synthesis.

Alternatively, the insulin stimulation of myogenesis may be a consequence of alterations in cyclic nucleotide metabolism, since insulin is known to affect cyclic AMP synthesis[13] and degradation[14] and cyclic GMP levels[6] in different cell types. Therefore it is attractive to suppose that such effects account for the stimulation of myogenesis in L_6 myoblasts reported here, and perhaps for the insulin-promoted differentiation of mammary gland in organ culture[15], and of primary cultures of lens epithelium[16]. In the case of L_6 myoblasts, it is worth noting that insulin improves both the reproducibility and synchrony of fusion, and allows myogenesis to be studied in a defined serum-free medium.

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Sickle cell resistance to *in vivo* hypoxia

THE rapid disappearance from circulation of human erythrocytes transfused to rats can be prevented if the animals are pretreated with ethyl palmitate (EP) and a cobra venom factor (CVF)[1]. The improvement in human erythrocyte survival in these animals is due to EP-induced impairment of reticuloendothelial function[2] and to suppression of intravascular haemolysis through CVF inhibition of the third component of complement[3]. The halflife of normal human erythrocytes in the blood of untreated rats is less than 15 min, but is about 30 h in the EP and CVF treated rats[4].

Application of this animal system to the study of human sickle cell anaemia erythroctyes (sickle cells) has verified many of their known survival characteristics in man[5-7]. These include a short intravascular survival, an improved survival with *in vivo* hyperoxygenation and an improved survival following *in vitro* cyanate treatment[8,9]. Human sickle cells transfused into rats exposed to widely divergent concentrations of oxygen seem to consist of two populations with regard to their *in vivo* sensitivity to oxygen changes. The circulation of a minor population of sickle cells is dependent on the concentration of oxygen the rats breathe, as shown by rapid increases and decreases in circulating cells with increases and decreases in the amount of oxygen administered to the rats (O_2-sensitive cells)[8,10]. In contrast, the majority of sickle cells seems to be relatively insensitive to the levels of hypoxia attainable *in vivo*. They continue to circulate after the initial rapid removal of the oxygen-sensitive cells in spite of exposure to hypoxic atmospheres barely compatible with life. The proportion of sickle cells which is oxygen sensitive is not influenced by the total number of sickle cells transfused (O.C., S.C.F., and G.O., unpublished). Oxygen-sensitive sickle cells are most readily demonstrated when the animals breathe 100% O_2 followed by 7–8% O_2 in short alternating periods. In these conditions the ^{51}Cr survival curve of the transfused sickle cells assumes a characteristic 'saw tooth' pattern with peaks during hyperoxia and valleys during hypoxia[10].

Here we report that separation of hypoxia-resistant sickle cells is possible if blood from a rat previously transfused with sickle cells is removed during hypoxia and then retransfused into another rat. Presumably all of the human sickle cells in circulation in the blood of donor animals during hypoxia are insensitive to a low pO_2 after the sensitive population has been sequestered. Figure 1 shows that in the recipient animals the

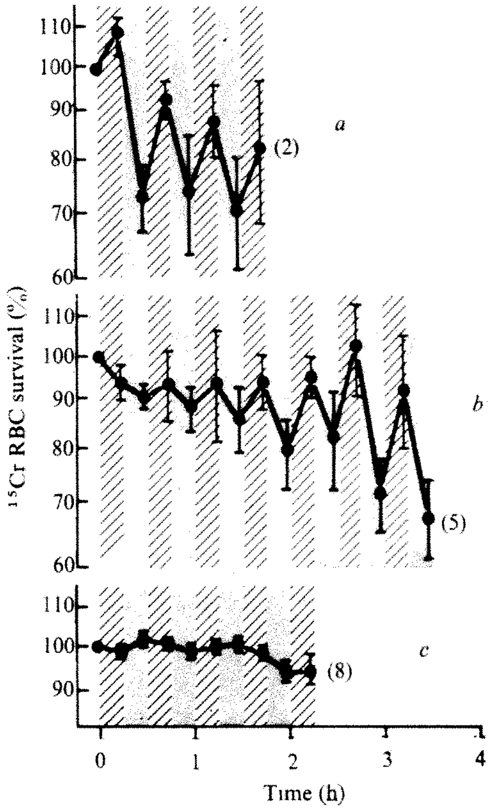

Fig. 1 Human sickle cell resistance to O_2-dependent circulatory changes after their removal from hypoxic rats and retransfusion into separate animals. Each rat was injected with 0.5 g kg^{-1} of EP and with 10 units of CVF 2 h before transfusion. Three donor animals then were transfused with 4 ml of a 50% saline suspension of ^{51}Cr-labelled erythrocytes (RBCs) from one of three patients with sickle cell anaemia. After a 20–60 min exposure of the donor animals to 7.5% O_2 in N_2 5 ml of rat blood—containing transfused ^{51}Cr human sickle cells—were removed by cardiac puncture. The RBCs were washed with physiological saline, adjusted to a 50% suspension in saline and then 1–3 ml were retransfused into recipient animals. Following this, all rats were exposed to a minimum of three hyperoxia–hypoxia cycles each consisting of 15 min of 100% O_2 followed by 15 min of 7.5% O_2 in N_2. The ordinate measures ^{51}Cr RBC survival expressed as % activity found in the rats' blood immediately before exposure to the hyperoxia–hypoxia cycles. The abscissa measures time (h) after start of the first hyperoxia–hypoxia cycle. Points in the graph represent means and bars ± 1 s.d. of the values for each patient study. The number of rats used in each group is shown in parentheses. *a*, Transfused sickle cells showing that the sickle cells remaining in the two donor animals that survived cardiac puncture exhibited characteristic O_2-dependent circulation during each hyperoxia–hypoxia exposure. *b*, Retransfused sickle cells (previously obtained from hypoxic donor animals) did not show this behaviour initially. After the third cycle, an O_2-sensitive population developed in the retransfused sickle cells. *c*, The survival in the rats' blood of ^{51}Cr-labelled control human RBCs obtained from two individuals without haematological abnormalities was not influenced by oxygen changes. Stippled areas, hypoxia; cross-hatched areas; hyperoxia.

survival curves of retransfused sickle cells obtained from hypoxic donor animals do not exhibit the saw tooth pattern during the initial alternating periods of hyperoxia and hypoxia. The sickle cells remaining in the donor animal (transfused sickle cells) continue to show this response. Sickle cells removed from donor animals while they breathe 100% O_2 and retransfused into recipient animals (not shown in the figure) retain an O_2 sensitive population. Continuation of the hyperoxia–hypoxia cycles in the recipient rats with O_2 insensitive retransfused sickle cells eventually results in a survival pattern consistent with the development of a population of sickle cells which is oxygen sensitive (Fig. 1). The proportion of O_2-sensitive sickle cells to total sickle cells is similar to that in the original population. Wide changes in O_2 tension seem to be necessary for the appearance of an O_2-sensitive population of sickle cells in the recipient animals. This statement is based on the observation (O.C., S.C.F., and G.O., unpublished) that the O_2-insensitive retransfused sickle cells in rats maintained on room air will remain resistant to O_2 changes until they have been subjected to several hyperoxia–hypoxia cycles.

The physical separation of hypoxia resistant sickle cells from the total population confirms the heterogeneity of human sickle cells with regard to their oxygen dependent circulation in this animal model. The temporary loss of O_2-sensitive sickle cells from the animals' circulation during exposure to low O_2 tension is best explained by *in vivo* sickling and trapping of sickled erythrocytes in the animals' microvasculature. This explanation is supported by our finding in this animal model of hypoxia sensitive erythrocytes not only from patients with sickle cell anaemia but also from patients with sickle-thalassaemia and with sickle-Hb C disease[4]. In contrast, none of eight individuals with sickle trait studied thus far has shown this type of response[11].

The observations reported here demonstrate that a proportion of O_2-insensitive sickle cells can be transformed into an oxygen sensitive population when challenged with repeated hyperoxia–hypoxia cycles. Our findings suggest that repeated sickling may be an important factor in the production of cells that do not circulate during hypoxia. Direct extrapolation of animal data to the human situation must be viewed with caution. Nevertheless, it is possible that vaso-occlusive episodes in patients with sickle cell anaemia may be a function of the relative number of sickle cells that are oxygen sensitive with regard to their capacity to circulate. The isolation and study of any oxygen resistant population may help to determine those factors that protect sickle cells from sequestration during *in vivo* hypoxia.

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Expression of the dystrophia muscularis (*dy*) recessive gene in mice

STUDIES *in vitro* of mutant gene expression in murine muscular dystrophy now seem to have reached an impasse. For 15 years there was only one species of dystrophic mouse[1], the Bar Harbor 129 ReJ strain (denoted *dy*/*dy*). Then in 1970, a second strain BH WK ReJ (denoted dy^{2J}/dy^{2J}) was reported[2] which was described as being similar in all histopathological respects to the allelic *dy*/*dy* mutant except that it showed a slower progression of muscle necrosis. The new mutant gene was transferred to another mouse strain C57Bl/6J for commercial retail. The new strain, offering many advantages over the original strain, has been readily adopted for study by research workers; certain findings obtained with this strain have added confusion to a difficult problem simply because it is not the same as the original strain.

Over the past four years I have been growing dystrophic (*dy*/*dy*) muscle *in vitro*, and have noticed that muscle explants from crush lesions in dystrophic (*dy*/*dy*) muscle did not form multinucleate myotubes, whereas muscle explants from crush lesions in normal muscle readily did so. I report here some brief details of this work (previously described in refs 3 and 4), and include the results of some preliminary experiments using dystrophic (dy^{2J}/dy^{J2}) mice which suggest that allelic mutant genes do not have the same phenotypic expression.

Crush lesions were made in the triceps and quadriceps muscles of normal Bar Harbor 129 ReJ+/+, dystrophic 129 ReJ *dy*/*dy*, and dystrophic C57Bl/6J dy^{2J}/dy^{2J} mice under pentobarbital anaesthesia. Four days after the lesion was inflicted the mouse was killed by cervical dislocation and the lesion zone was removed under sterile conditions for tissue culture. The excised regions were dealt with separately. The tissue was chopped and the explants set up in coded Pulvertaft[5] chambers or 50-mm Petri dishes. The culture medium was 10% EE50, 10% horse serum and 80% Eagle's MEM with streptomycin and penicillin.

Each day, over a period of 8 d, all the coded cultures were assessed microscopically using differential interference optics. The assessment was carried out blind and the results are therefore unbiased. The total number of cultures was in excess of 100 for normal and *dy*/*dy*, and 40 for dy^{2J}/dy^{2J} mice.

Days 1, 2 and 3 showed essentially the same pattern of growth in all cultures. Proliferation and explant spreading were similar, and occasional cells with up to five nuclei were found. From day 4 to day 8 proliferation continued until the cultures became overgrown and degenerated. During this second period all normal cultures (Fig. 1*a*) and all dystrophic dy^{2J}/dy^{2J} cultures (Fig. 1*b*, *d*) developed multinucleate myotubes of considerable size. There was an indication, which could not be quantified, that explants of dy^{2J}/dy^{2J} quadriceps showed less multinucleation than triceps cultures from the same animal. All dystophic *dy*/*dy* cultures (Fig. 1*c*) showed associations of cells which have been named pseudostraps[4]. These are muscle strap-like or myotube-like aggregates of myoblasts which do not fuse. There were no multinucleate structures of any kind to be found. I believe that these results clearly demonstrate an abnormality of early myogenesis caused by the expression of the *dy*/*dy* gene, but no indication of any abnormality caused by the expression of the dy^{2J}/dy^{2J} gene.

The results clarify some of the conflicting evidence already produced. Three groups[6–8] have used organotypic methods of tissue culture combining foetal spinal cord sections with adult muscle. Paul *et al.*[7] were very careful not to draw any far-reaching conclusions from their work, recognising the genetic background on which it was based. But they did state that adult myogenic cells were in some way affected by the dystrophy (*dy*/*dy*) and were incapable of

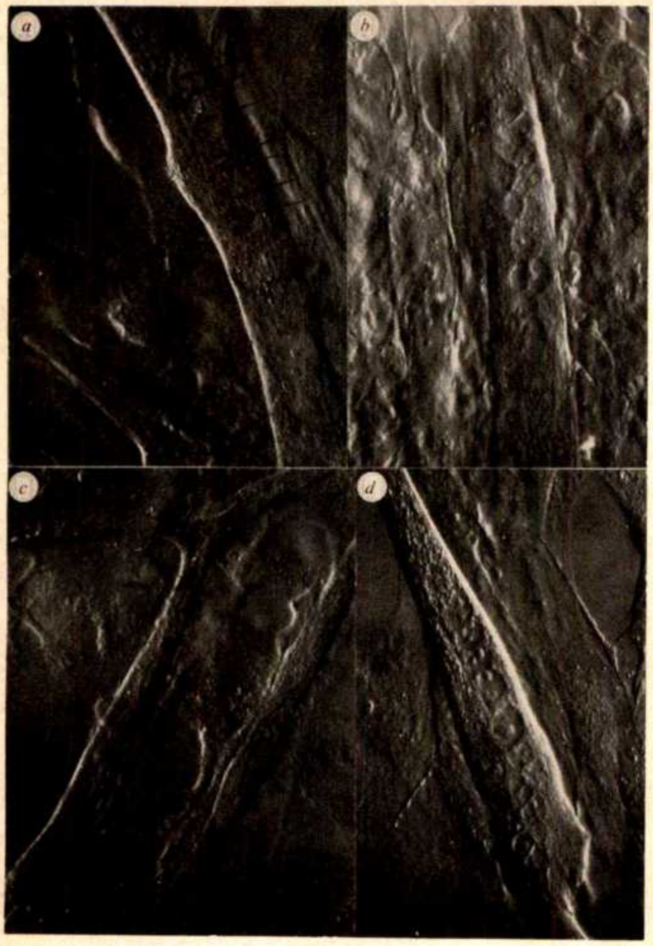

Fig. 1 Unstained, living cultures seen using differential interference optics (Reichert). They are representative of muscle formation *in vitro* from crush lesion explants. *a*, Part of a large multinucleate myotube showing a chain of central nuclei (arrows), normal +/+. *b*, Part of a myotube from dystrophic dy^{2J}/dy^{2J} quadriceps. c, Pseudostraps from dystrophic dy/dy muscle. Note the close associations of cells which always remain discrete. *d*, A clear multinucleate myotube from a dystrophic dy^{2J}/dy^{2J} triceps culture. All photographs were taken at 7 d *in vitro*, under identical conditions.

regeneration. Hamburgh *et al.*[8] came to the conclusion that muscle from dystrophic (dy^{2J}/dy^{2J}) mice exhibited a capacity to regenerate similar to normal muscle. Gallup *et al.*[6], using both strains of mice, concluded that the regeneration of normal and dystrophic muscle is dependent on the genotype of the cord section with which it is coupled. Peterson[9], using mouse chimeras (SWV+C57Bl/6J dy^{2J}/dy^{2J}), was led to suggest that the presence of dystrophic nuclei did not affect myogenesis in any way. My results are essentially similar to those of Paul *et al.*[7] and Hamburgh *et al.*[8] I cannot, however, agree with any of the findings or conclusions of Gallup *et al.*[6]. Finally, I am in agreement with Peterson[9] that dy^{2J}/dy^{2J} muscle behaves in the same way as normal muscle with regard to myogenesis.

Therefore I would submit that the recent study of murine dystrophy has been based on the false assumption that allelic mutant genes have the same phenotypic expression. Consequently, if researchers into muscular dystrophy are to continue to use the mouse as an experimental animal they must take care to specify the strain of animal used before drawing up conclusions relating to the pathogenesis. These findings suggest that the causal lesion of the dy/dy dystrophic mutant lies in the muscle cell; this seems unlikely to be the case in the so-called (dy^{2J}/dy^{2J}) dystrophic strain.

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Growth of human muscle spindles *in vitro*

STRIKING histological, histochemical and physiological differences between intrafusal and extrafusal muscle have been observed in a large number of species. Enzyme profiles, for instance, of intrafusal muscle not only fail to reflect the extrafusal pattern but show considerable interspecies variation[1,2].

The precise relationship of genetic, neural and environmental influences and their role in determining the peculiar properties of intrafusal muscle, is uncertain. There is strong evidence that innervation is essential for the normal ontogenetic development of spindles[3] and neural control of the histochemical properties of fully differentiated extrafusal muscle has been shown to be considerable[4]. Spindles denervated at maturity, however, are remarkably resistant to change.

We considered that an investigation of the growth and differentiation of intrafusal muscle *in vitro*, thus isolated from its normal neural and environmental influences, might elucidate the role of genetic factors in determining its characteristics *in vivo*. It was of particular interest to examine the behaviour of human tissue in this context in view of the large number of genetically determined muscle disorders and the relative lack of information concerning the pathological role of the spindle.

The regenerative capacity of skeletal muscle following injury, disease, or experimentally-induced trauma is considerable[5-8] and is, to some extent, reflected by the growth and differentiation of muscle explants in culture. Normal and diseased adult human muscle has been successfully grown *in vitro* by several workers[7-9], but none has specifically investigated the growth of intrafusal muscle. Whether spindles possess a similar regenerative potential and to what extent intrafusal muscle retains its identity in the simplified conditions of tissue culture is as yet unknown.

The varied findings of other workers in the field indicate that the culture employed may itself influence results and, for valid comparisons to be made between the behaviour of different tissues *in vitro*, must be a controlled factor. For this reason, in our study, explants of both extrafusal and intrafusal muscle from each specimen were cultured in identical conditions.

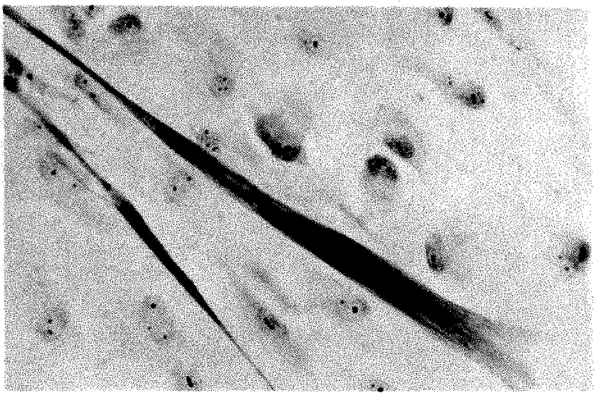

Fig. 1 A multinucleate myoblast from a culture of intrafusal muscle after 36 d *in vitro* (×280.80).

Muscle specimens (commonly of palmaris longus) were obtained from patients undergoing diagnostic, motor-point muscle biopsy. Adjacent specimens in each case were examined by routine histological and histochemical procedures and cases in which the results of these investigations were found to be within normal limits were used in this study.

The specimen, approximately 2 cm×0.5 cm was placed in a Petri dish containing Eagles minimum essential medium (MEM) with 100 U ml^{-1} penicillin and streptomycin. Any spindles present were dissected out from the surrounding tissue under a microscope, care being taken to isolate the spindle completely from the extrafusal fibres. The outer layers of the spindle capsule were removed and the spindle cut transversally to form explants approximately 1 mm long. The explants were placed on coverslips coated with reconstituted rat tail collagen and covered with a thin clot of chicken plasma. They were then placed in test tubes with 3 ml of a nutrient medium consisting of 80% MEM, 10% horse serum and 10% chick embryo extract. Each tube was sealed with a silicone rubber bung, placed on a tube roller and incubated at 37° C. Small pieces of extrafusal muscle (approximately 1 mm cube) were set up in a similar manner. The medium was changed once a week and living cultures were examined from time to time using the Maximow slide system and phase contrast microscopy. All the above procedures were carried out aseptically. At intervals between 20 and 70 d after setting up, suitable cultures were fixed and stained with PTAH.

Fifteen stained cultures of normal intrafusal muscle have so far been obtained, these being derived from nine different patients and representing less than half the number of normal intrafusal cultures initially set up, the remainder have failed to grow. In all cases parallel extrafusal cultures were also obtained.

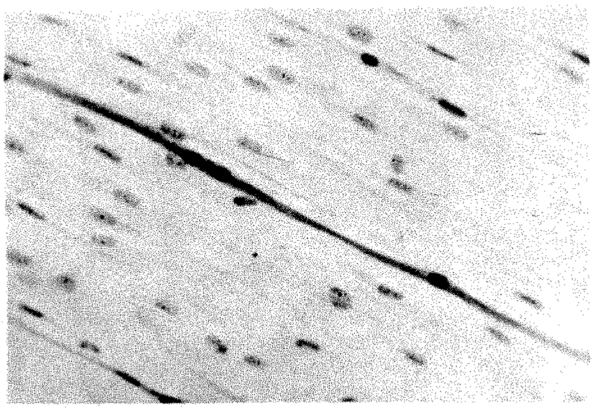

Fig. 2 A myotube from a culture of intrafusal muscle after 55 d *in vitro* (×176.80).

The first growth of intrafusal muscle is observed between 10 and 25 d *in vitro* and may precede signs of growth in parallel extrafusal cultures by several days. Microscopic examination at this early stage reveals, however, only mononucleate cells and it is likely that a large proportion of these are fibroblasts derived from the relatively large amount of capsular connective tissue inevitably left within the explant.

Immediately following the initial proliferation of mononucleate cells, typically elongated and spindle-shaped in appearance, the majority of cultures show in addition a variable number of multinucleate cells. Figure 1 shows a multinucleate myoblast in an intrafusal culture at 36 d; such cells are visible within the first few days of growth following the lag period and continue to be found in cultures 70 d old. The cells show extreme variability of size, shape and number of nuclei and in older cultures there is, in addition, an increasing proportion of myotube forms, elongated syncitia in which some or all of the nuclei become separated or aligned in chains (Fig. 2). At a later stage peripherally situated nuclei are seen in many of these cells.

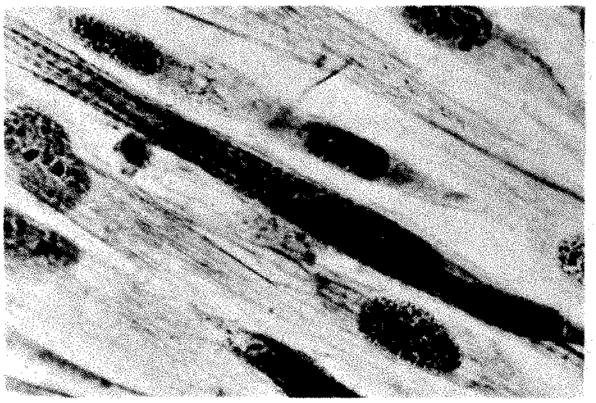

Fig. 3 Cross striations in a myoblast from a culture of intrafusal muscle after 70 d *in vitro* (×728).

Cross striations were observed in a small minority of myoblasts and myotubes in both intrafusal and extrafusal cultures (Fig. 3). They were seen in both mononucleate and multinucleate cells and when fully differentiated Z lines. A and I bands and occasionally H zones were visible, but at no time during the observation of any of our living cultures was spontaneous contraction observed.

Owing to the small number of cultures so far obtained and the widely varying appearance of the growing cells, statistical examination of results has not yet been undertaken, though some measurements of size have been made. In a culture of intrafusal muscle at 31 d in which the lag period was 20 d (that is, after 11 d growth) myotubes reached a maximum length of 720 μm. This value became 800 μm in another culture after 18 d growth. (These cells were less than 20 μm in width.) Another intrafusal culture after 35 d growth produced many shorter, broader myoblasts, commonly 100 μm long and 50–60 μm wide, containing numerous densely packed nuclei. The longest cell reached only 400 μm.

In parallel extrafusal cultures, cells also showed extreme variation of form, but in all cases measured, the maximum length of myotube exceeded the value for intrafusal cultures, for example up to 1,100 μm after 18 d growth. This observation, however, may only reflect the considerably larger number of extrafusal explants obtainable from any specimen and their relatively greater mass.

It is apparent that intrafusal muscle possesses a considerable regenerative capacity under tissue culture conditions and there is little doubt that regeneration of muscle is a function of satellite cells, of which there are many within muscle spindles[9]. No ultrastructural differences can be discerned between extrafusal and intrafusal satellite cells and our preliminary observations indicate that the growth and differentiation of intrafusal muscle *in vitro* closely parallels the behaviour of extrafusal muscle. If confirmed by further investigation, for instance of the histochemical and ultrastructural properties of the growing cells, it would suggest that a critical factor for the differentiation of muscle spindles is trophic rather than genetic and is absent under these conditions. A strong possibility is that such a factor derives from the neural influence and it is interesting to consider to what extent this could be supplied in a tissue culture system and how much its precise nature may be analysed by this means.

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Multiple control mechanisms underlie initiation of growth in animal cells

THE understanding of the control of cell proliferation and differentiation requires the unravelling of the early events that trigger the initiation of growth. An *in vitro* system to study the initiation of growth is provided by the mouse 3T3 cell line. These cells exist in two alternative growth states: either reversibly arrested in the G_0 phase of the cell cycle or in active proliferation. When resting 3T3 cells are exposed to fresh serum they recommence DNA synthesis and cell division[1]. Functional membrane changes are among the earliest events associated with the reinitiation of growth[2]. Within minutes of serum addition, the rate of transport of inorganic phosphate, nucleosides and glucose is increased several fold[2–6], while cyclic AMP, which has been implicated in the regulation of growth of cultured fibroblasts, shows an opposite change[7–11].

The coordinated expression of these membrane changes raises the possibility that some of these events are cause-effect related to each other[12,13]. Two different hypothesis concerning this important problem have evolved. A unifying view holds that cyclic AMP acts as a pleiotypic modulator that regulates the overall rates of all the membrane transport systems affected by changes in cell growth[5,14]. The alternative view proposes that cyclic AMP might control some specific changes in membrane transport but not a general change in cell permeability [10,11]. The first hypothesis predicts that there should be a high degree of coupling between the various biochemical changes stimulated by serum or insulin while the second envisages the changes as dissociable, since they are supposed to be controlled by several distinct mechanisms.

The experiments presented here were conducted to differentiate between these alternative possibilities. Our results substantially support the hypothesis of specific changes and suggest that at least three distinct mechanisms of control of membrane transport operate when growth is initiated.

Uridine and phosphate transport systems respond to separate control mechanisms as shown by the differential effects produced by the addition of ouabain, cycloheximide and cyclic AMP elevating agents. Typical experiments illustrating this point are shown in Fig. 1. Ouabain, an

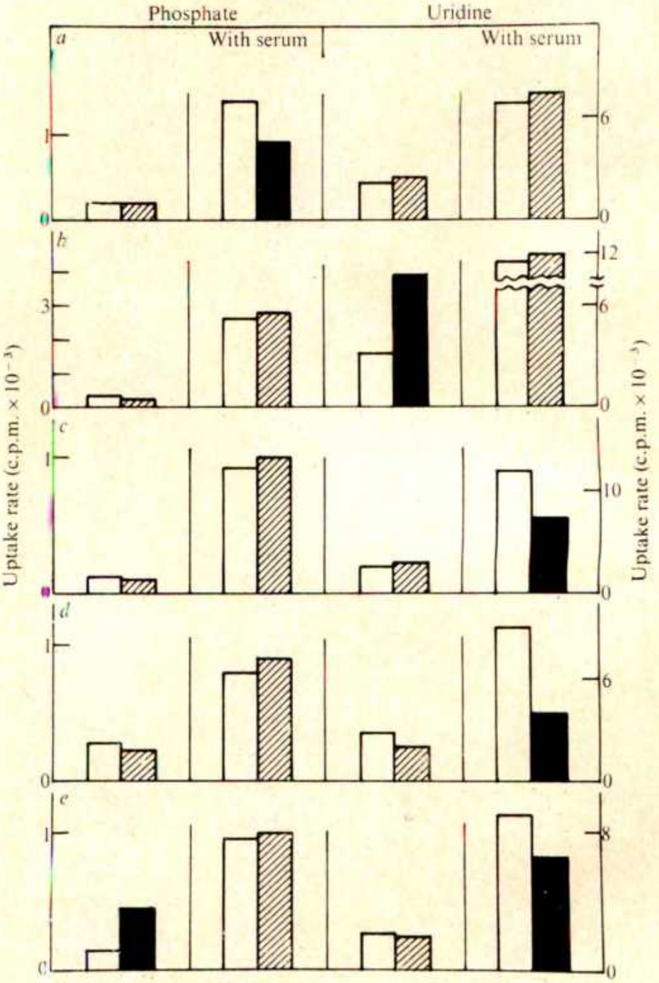

Fig. 1 Differential effects of, *a*, 1 mM ouabain; *b*, 10 μg ml^{-1} cycloheximide; *c*, 1.5 mM theophylline; *d*, 0.2 mM SQ 20006 and *e*, 40 μg ml^{-1} PGE$_1$ on uridine and phosphate uptake rates. Swiss 3T3 fibroblasts were maintained in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum, 100 units ml^{-1} penicillin and 100 mg ml^{-1} streptomycin in Nunc Petri dishes. Transport was measured on cells attached to 30 mm dishes. Confluent cultures were used usually 3 d after the last change of medium. First, the cells were washed with phosphate-free medium and incubated in this medium with (hatched bars) or without the above mentioned compounds at the concentrations indicated. After 10 min, dialysed foetal calf serum (10% final concentration) was added to some dishes and the culture incubated for additional 20 min. Then, the cells were labelled with ^{32}P-phosphate (75 μCi μmol^{-1}) 3.75 μCi ml^{-1}) and ^{3}H-uridine (29 mCi μmol^{-1}, 2.5 μCi ml^{-1}) for 5 min. Uptake into acid-soluble pools and all other materials and methods were measured as described previously[10–11]. Each bar represents the average of two transport determinations. Each experiment was repeated at least three times. The treatments that produced effects were shown with dark bars.

inhibitor of the (Na^+, K^+) APTase, significantly decreases the activation of phosphate transport produced by serum but has no effect either on the increase of uridine uptake or on cyclic AMP levels. Conversely, elevation of the intracellular levels of cyclic AMP by prostaglandin E_1 (PGE_1), theophylline or 1-ethyl-4-hydrazino-1H-pyrazolo-(3,4-b)-pyridine-5-carboxylic acid, ethyl ester, hydrochloride (SQ 20006) (ref. 15), depresses the activation of uridine transport by serum while the stimulation of phosphate uptake is unaffected. In the absence of serum, PGE_1 stimulates the uptake of phosphate but not that of uridine. Further, cycloheximide added in the absence of serum activates uridine uptake, in agreement with earlier results[5,12] but it does not modify the basal uptake level of phosphate. In addition, when the uridine transport rates determined in the presence of different compounds are plotted as a function of cyclic AMP levels measured in parallel cultures, an inverse correlation is obtained. On the other hand, no correlation is obtained when phosphate transport rates are plotted as a function of cyclic AMP concentration (data not shown). All these results show that the early transport changes of uridine and phosphate are dissociable.

In addition to stimulating phosphate and uridine transport, serum brings about a large increase in 2-deoxyglucose uptake in resting 3T3 cells[4-6]. A third level of transport control was identified when this phenomenon was carefully examined. The activation curve as a function of time is clearly biphasic (Fig. 2). The early phase was unaffected by the presence of cycloheximide while the second was completely prevented by this inhibitor. Other experiments reveal that the kinetic parameter modified as a function of time is the maximum velocity (L. J. de A., and E. R., unpublished). Thus, in addition to changing rapidly the uptake of 2-deoxyglucose, serum seems to induce also the synthesis of new carrier molecules. For comparison, uridine and phosphate transport rates were measured in similar conditions (Fig. 2). A biphasic activation curve with a cycloheximide-sensitive second phase was also seen with phosphate uptake but not with uridine up to 5 h after serum addition.

The induction of the second phase of glucose transport is not triggered by a drop in cyclic AMP since SQ 20006 does not block it (Table 1) and insulin which decreased cyclic AMP in resting cells[7,8,10] and increases cyclic GMP in different tissues[16] does not induce it. Cyclic AMP, however, could modulate the activity of the transport system when it is already inserted in the membrane (Table 1) which may account for the rapid phase stimulated by serum.

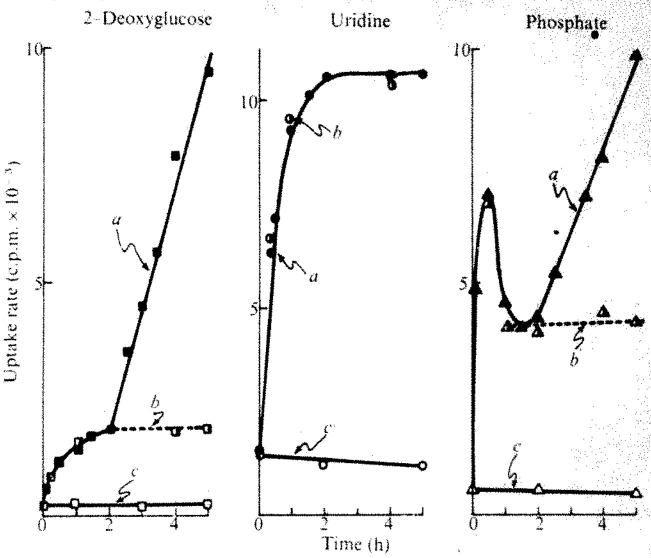

Fig. 2 Kinetics of the changes in 2-deoxyglucose, uridine and phosphate transport produced by the addition of fresh serum to resting cultures of 3T3 cells in the absence or presence of cycloheximide (10 μg ml^{-1}). The inhibitor was added at time 0. The uptake of ^3H-2-deoxyglucose was determined as follows: the cells were washed four times with prewarmed serum-free medium minus glucose and incubated for 10 min in this medium with or without dialysed serum or other additions. The cells were then exposed to labelled substrate (3 μCi ml^{-1}; 5×10^{-5} M) for 10 min. Uptake into the 5% TCA soluble fraction was linear for 15 min and was measured as described earlier[10-11]. The other determinations were carried out as indicated in the legend to Fig. 1. *a*, Serum; *b*, serum + cycloheximide; *c*, serum-free medium.

Table 1 Effect of SQ 20006 on the uptake of 2-deoxyglucose stimulated by serum*

	Additions		Uptake rate
	Serum	SQ 20006	(c.p.m. per dish)
A	–	–	320
	+	–	890
	+	+†	520
B	–	–	280
	+	–	7,751
	+	+†	4,256
	+	+‡	5,179
	+	+§	7,002

* Resting cultures of 3T3 cells were exposed to 15% foetal calf serum for 40 min (A) or 4 h 30 min (B). Then, the uptake of 2-deoxyglucose was measured as described in the legend to Fig. 3.

† Added at time 0.

‡ Added 4 h after serum.

§ Added at time 0 and removed 4 h later by washing four times with prewarmed medium.

The possibility of eliciting or inhibiting selectively some early biochemical events by using a variety of compounds clearly shows that these membrane changes are dissociable. All these results support the hypothesis that conceives distinct mechanisms of control switched on when growth is initiated and seem incompatible with a pleiotypic role for cyclic AMP in the control of all the changes in membrane transport produced by serum. In fact from the present and previous findings[10,11] it is possible to define at least three distinct mechanisms of control, that can be differentiated on the basis of temporal expression, dependence on cyclic AMP levels and on (Na^+, K^+) ATPase activity and requirements for protein synthesis.

The various metabolic changes studied here might be produced independently by different factors present in serum or by different chemical signals synthesised by the cells when exposed to a single factor. Another important question that can now be asked is whether or not all the biochemical changes stimulated by serum are required for the initiation of cell division. Our studies offer experimental basis for attacking these problems, the clarification of which will contribute substantially to our understanding of the initiation of growth.

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Control of cell division in yeast using the ionophore, A23187 with calcium and magnesium

A23187 is an ionophore which exhibits specificity for divalent cations and promotes their passive transport across biological membranes[1]. It therefore effects equilibration of divalent ions between the cell sap and the surrounding medium and counteracts any active accumulation of ions within the cell. Evidence, mainly from studies on sea urchin eggs, supports the hypothesis that calcium ions play an important part in bringing about cell division[2-4]. We have found that division in the fission yeast, *Schizosaccharomyces pombe*, is associated with a doubling in magnesium concentration within the cells[5]. It, therefore, seems likely that, if accumulation of divalent ions within the cell were an essential prerequisite of cell division, this should be inhibited by A23187 and cell division should not take place, and so we have investigated this possibility.

S. pombe, strain 132, was grown in the defined medium, EMM2 (ref. 6). Once the cells were growing exponentially and had reached a concentration of 10^6 cells ml^{-1} or more, 1.0 ml of the stock solution of A23187 was thoroughly mixed with 100 ml of culture. The stock solution was prepared by dissolving 0.5 mg A23187 in 0.05 ml of Analar acetone and adding 1 ml of absolute ethanol[7]. Subsequently, cell division was inhibited quite rapidly, only 10–15% of the cells dividing before inhibition was complete. The ethanol/acetone mixture on its own had no effect.

If cells which had been left in growth medium with A23187 as above for 3 h at 30° C were collected and immediately inoculated into fresh medium, synchronous division (cell plate index = 25–30%) ensued about 30 to 40 min after transfer. The next division was also synchronous. DNA measurements showed that the cells had been inhibited by A23187 in the G2 phase of the cycle. Following staining with Giemsa[8], up to 47% of the cells appeared to be binucleate. Cells with a complete cell plate, but in which division had not started as indicated by formation of a 'waist' at the cell plate, were taken as one cell. Thus, slightly more than half of the binucleate cells contained cell plates. Occasionally a cell was observed containing two cell plates close together and, in between, a piece of cytoplasm undergoing autolysis.

To define more precisely the point at which inhibition of division was occurring, a synchronous culture of *S. pombe* was established using selection of newly divided cells from a sucrose gradient[6]. Samples were taken from this culture at suitable intervals and A23187 added to give the same final concentration as above. These samples were left for 3 h at 30° C and the cell number measured. In the absence of inhibition, division should have occurred within this time. This was verified using controls without A23187 but with the ethanol/acetone solvent. Even with a sample of cells taken only 10 min before division could be detected, A23187 completely prevented division taking place. Thus the block to cell division must occur very close to the end of the cell cycle. Possibly the initial blockage is at a stage just before nuclear division, whereas any cell at a point after nuclear division and before cell division is blocked at other stages which are particularly sensitive to the changes brought about by the ionophore.

Table 1 Change in calcium and magnesium content of cells following addition of 5 μg ml^{-1} A23187 and incubation at 30° C for 3 h

Before addition of A23187	Ca (g per cell)	Mg (g per cell)
Supernatant liquid	3.0×10^{-14}	0.5×10^{-14}
Pellet	37×10^{-14}	1.3×10^{-14}
After addition of A23187 and incubation		
Supernatant liquid	5.0×10^{-14}	0.9×10^{-14}
Pellet	5.0×10^{-14}	0.6×10^{-14}

Since A23187 is supposed to make membranes permeable to divalent ions, it was decided to investigate in general terms what happened to calcium and magnesium within the cell. Samples of cells were taken before and after A23187 treatment in EMM2 for 3 h as described above. These samples were quickly washed twice in glass-distilled water and homogenised in 5 ml glass-distilled water in an Eaton press[9]. The homogenate was centrifuged at 40,000g (R_{av}) for 30 min. The supernatant liquid was analysed without further treatment, using atomic absorption spectrophotometry to measure calcium and magnesium. The pellet was solubilised by digestion overnight at 40° C with 3 ml of a 1:2 (v/v) mixture of 60% perchloric acid and 30% hydrogen peroxide. The resultant solution was diluted to 10 ml with glass-distilled water and analysed for calcium and magnesium in the same way as the supernatant. The results obtained are shown in Table 1. It is clear that, following A23187 treatment, calcium in the pellet has dropped dramatically (sevenfold) while magnesium has dropped by half. The supernatant values have increased, but to a lesser extent. Since the pellet almost certainly contains mitochondrial vesicles, and there is evidence from other eukaryotic cells that mitochondria accumulate calcium and, to a must lesser extent, magnesium[10], this may reflect leakage of ions from the mitochondria brought about by A23187. The supernatant levels would then reflect altered steady state concentrations in the cytosol. Overall, the total calcium content of the treated cells has dropped to 25% of that of untreated cells while the magnesium content has dropped to 80%. This probably reflects the differential gradients for these ions between the cell and the medium where the calcium concentration is 0.07 mM and the magnesium concentration 2.5 mM.

Finally, we attempted to reverse the effects of A23187 by adding calcium and magnesium to cells which had been treated with A23187 in EMM2 as previously described for 3 h. Increasing the calcium concentration of the medium tenfold (to 0.7 mM) and the magnesium concentration likewise (to 25 mM) enabled division of about 90% of the cells to occur between 15 and 20 min after adding the ions as their chloride salts. This is somewhat faster than the recovery when the cells are transferred to fresh medium (see above) but no further divisions ensue whereas the cells in fresh medium recover completely.

Similar results to the above have been obtained for the budding yeast, *Kluyveromyces fragilis* (Penman, unpublished). We therefore conclude that a pool of calcium and magnesium is essential for cell division in yeast. It may be required to promote the breakdown of microtubules involved in nuclear division and cell plate formation[11] and this possibility is being investigated.

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Antigen of mouse bile capillaries and cuticle of intestinal mucosa

CELL surface antigens have been isolated by solubilisation and fractionation of membranes[1-3]. Here we present data on the membrane antigens of mouse liver cells soluble in the nonionic detergent Triton X-100.

Mouse liver cell ghosts were obtained as in ref. 4. Triton fractions (TF) from ghosts and from whole mouse liver cells were obtained as in ref. 5. TFs were also prepared from mouse kidney, intestine, spleen and the mouse ascitic hepatoma 22-a.

Liver TFs were analysed by gel double diffusion[6] and by immunoelectrophoresis[7] using rabbit antisera to liver cell ghosts. These sera do not react with soluble antigens of the liver cell and in immunofluorescent studies they stain predominantly the plasma membranes of parenchymal cells[8] (Fig. 1*a*). Before analysis all the TFs were concentrated 10–20 times and anti-ghost sera were concentrated four times. Each TF of mouse liver was checked by the precipitin reaction with 40 samples of rabbit anti-ghost sera obtained from different animals and in different cycles of immunisation.

In all TFs of mouse liver cells or ghosts studied, six antigens were found which were precipitated by the analysed rabbit anti-ghost sera. These antigens were presumably membrane antigens. One of them, called here BCCA (bile capillary-cuticular antigen) was regularly found in each liver TF whereas the other five antigens were detected in only some of the TFs. The latter five antigens are probably present in too small amounts to be detected consistently. It seems however, that modifications in the method of preparation of TF will eventually overcome these variations. Using immunoprecipitation and indirect immunoautoradiography the distribution of BCCA in different mouse organs and in the ascitic hepatoma 22-a was studied. BCCA is the dominant antigenic component of the TFs from the liver cells and ghosts and it is stable during storage. It is detected in considerable amounts in TFs of the mouse kidney. In TFs of the ascitic hepatoma 22-a it is determined only by immunoautoradiography. It is not detected in concentrated saline extracts of mouse liver, kidney spleen or intestine by either the agar precipitation test or immunoautoradiography.

BCCA was partly purified by agar-gel preparative electrophoresis[7]. The combined and concentrated TFs of the mouse liver were electrophoresed in buffered 1% agar (0.5 N barbital buffer, *p*H 8.6). The electrophoretic fractions (in the region of β_1-globulin) with maximal BCCA concentration and minimal amounts of other antigens were collected and combined.

This partially purified BCCA and the anti-ghost serum containing antibodies predominantly to BCCA were used for the preparation of monospecific antibodies to BCCA[10].

The cell localisation of the BCCA was studied by indirect immunofluorescence. The acetone-fixed cryostat sections of mouse organs were treated with monospecific antibodies to the BCCA and then by FITC-labelled purified donkey antibodies to rabbit IgG. The specificity of the fluorescence was checked by fluorescence inhibition.

Distinct, bright fluorescence was found on the liver cell surface surrounding the bile capillaries between two hepatocytes (Fig. 1*b*). The Kupfer cells, the epithelium of the bile duct and the vascular endothelium were not stained. The fluorescence of bile capillaries disappeared after the absorption of the monospecific antibodies to the BCCA with the BCCA-containing electrophoretic fraction. The native anti-ghost serum used for the preparation of the BCCA monospecific antibodies also selectively lacked the ability to stain bile capillaries after the absorption with the electrophoretic fraction containing BCCA (Fig. 1*c*).

In the sections of intestine (Fig. 1*d*) the monospecific antibodies to the BCCA stained the cuticle of the mucosa epithelial cells of villi and crypts. This distinct cuticular fluorescence, at different intensities, was observed for the whole length of the intestine from the duodenum to the rectum. The other morphological structures in the sections of the intestine were not stained. The cuticular fluorescence disappeared after the absorption of the monospecific antibodies to BCCA with TFs of the liver or intestine.

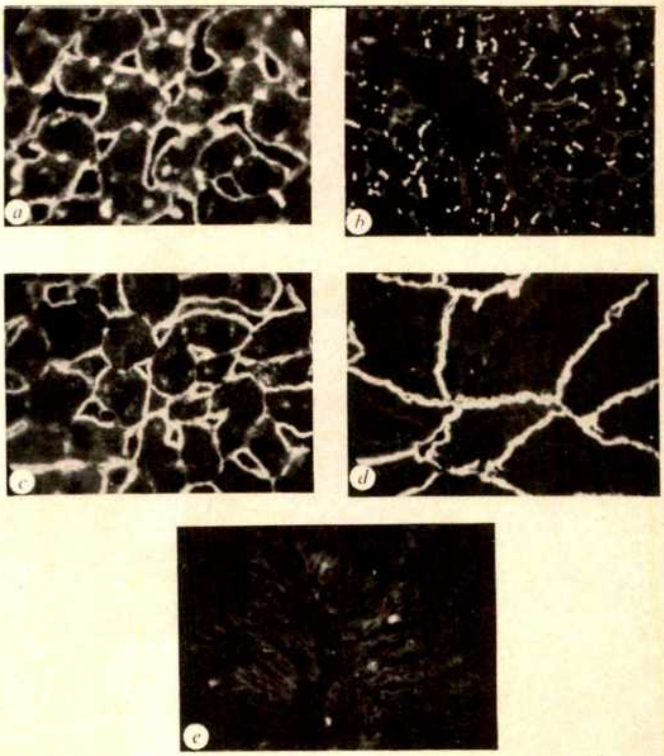

Fig. 1 *a*, Mouse liver section treated with native antiserum to liver cell ghosts. × 162. *b*, Mouse liver sections treated with monospecific antibodies to the BCCA. × 72. *c*, Mouse liver section treated with antiserum to liver cell ghosts absorbed by the partially purified BCCA (electrophoretic fraction). × 162. *d*, Sections of mouse intestine and *e*, stomach treated with monospecific antibodies to BCCA. × 162.

The monospecific antibodies to BCCA did not stain sections of the mouse oesophagus, stomach, kidney, spleen, pancreas and sections of the solid variant of the mouse ascitic hepatoma 22-a (the ascitic hepatoma, inoculated subcutaneously) (Fig. 1*e*).

So, BCCA is specifically localised in the bile capillaries of the mouse liver and the cuticle of the intestinal mucosa epithelial cells. A small amount of BCCA (or an antigen with a similar determinant) is also found on the plasma membrane of each liver cell. The absence of BCCA in the cytoplasm of liver cells suggests that it is synthesised on the cell surface.

The specific antigen of rat liver bile capillaries was described by Sulitzeanu *et al.*[12]. They also found it in kidney proximal tubule cells. Another antigen of rat bile capillaries has been reported[13]. The authors considered this antigen to be organo-specific but they did not demonstrate its absence in the intestine and other rat organs.

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Ultrastructural analysis of toxin binding and entry into mammalian cells

VERY low concentrations of phytotoxins can kill mammalian cells, and some of them, such as ricin and abrin, isolated from *Ricinus communis* and *Abrus precatorius*, have been reported to suppress protein synthesis in cells [1–3] and cell-free systems[4,5]. Ricin inhibits peptide chain elongation by catalytically inactivating the 60S subunits of ribosomes[5–7]. It is a galactose-binding glycoprotein of molecular weight 60,000, containing two nonidentical subunits linked by disulphide bridges[8–11] and seems to be identical to the lectin *R. communis* agglutinin II (RCA_{II}) isolated by affinity chromatography[8–12]. Ricin, or RCA_{II}, has been used to suppress the growth of ascites tumour cells *in vivo*[13], and as a cell surface probe for oligosaccharide receptors[14–17] containing terminal D-galactose or N-acetyl-D-galactosamine-like saccharides[11,14,18,19]. I have found that RCA_{II} enters mammalian cells by endocytosis (similar to the findings of Oliver *et al.*[20]) and is then released into the cytoplasm, where it apparently acts directly on protein synthesis.

Although ^{125}I-labelled RCA_{II} binds rapidly to mammalian cell surfaces[14,21] (saturation < 10 min at 5° C)[14], there is a significant lag before protein synthesis is inhibited (> 30 min at 37° C)[21,22]. This suggests that events subsequent to cell binding are essential for RCA_{II} toxicity[21,22]. Also, the toxic action of RCA_{II} can be prevented by addition of lactose[21] or antitoxin[22] within 15–30 min. In cell-free systems RCA_{II} suppresses protein synthesis almost immediately ($\approx$1–5 min)[4,6,21], and this action is not sensitive to RCA_{II} saccharide inhibitors[21]. To investigate the ultrastructural events subsequent to cell binding I have used an electron-dense ferritin-conjugate of RCA_{II} (Fer-RCA_{II})[23,24].

Fer-RCA_{II} was synthesised as described before[23–25] and purified by affinity chromatography on Biogel A-1.5 m[24]. After elution of column-bound Fer-RCA_{II} by 0.2 M D-galactose, the conjugate was dialysed extensively. Affinity purified Fer-RCA_{II} was active in suppressing murine 3T3 fibroblast protein synthesis, and the inactivation required much more time than required for simple binding to the cell surface (Table 1). The Fer-RCA_{II} conjugate was not as active in suppressing protein synthesis as native RCA_{II}; this might reflect some inactivation during the coupling process or a steric interference of ferritin with the enzymatic properties of RCA_{II}. But, as with RCA_{II}, the conjugate was blocked by the addition of D-galactose to the incubation medium (Table 1). To study the ultrastructural interaction of Fer-RCA_{II} with cells, the conjugate was added to 0.02% EDTA-dissociated, washed, confluently grown murine 3T3 cells suspended in Dulbecco's modified Eagle's minimal essential medium[26] (DMEM) containing 5% calf serum for various times at 4°, 20° or 37° C. Alternatively, cells were pulsed with Fer-RCA_{II} for 5–15 min at 4° C, washed by centrifugation and suspended in DMEM and then incubated further at 37° C for different times. Controls were labelled identically except that 0.1M D-galactose was present in the labelling and wash solutions. In some of the samples D-galactose was added at specific times during Fer-RCA_{II} labelling. Incubations were terminated by washing the cells in fresh DMEM and then adding 1.5% glutaraldehyde in sodium phosphate-buffered saline (*p*H 7.2; 310 mosmol). Fixation proceeded for at least 1 h, the first 30 min at the incubation temperature. The cells were washed again and postfixed in 1% osmium tetroxide in 0.1 M sodium phosphate buffer, *p*H 7.2, for 1 h at 20° C, washed, dehydrated in ethanol-propylene oxide and embedded in Epon 812. Thin sections were stained with 1% uranyl acetate and observed in a Hitachi HU-12 electron microscope.

3T3 cells labelled with Fer-RCA_{II} for 5–30 min at 0°–4° C or 5–10 min at 20°–37° C before fixation showed continuous labelling of RCA_{II} receptors, exclusively at the cell surface (Fig. 1*a*). Labelling was specific as D-galactose inhibits conjugate binding (Fig. 1*b*), as previously demonstrated[24]. Labelling at higher temperatures (20°–37° C) resulted in eventual lectin-induced receptor clustering of some but not all RCA_{II} receptors and subsequent endocytosis. At 37° C this sequence of events was more rapid than at 20° C, and significant endocytosis was seen within 60 min (Fig. 1*c*). With times greater than 60 min at 37° C most Fer-RCA_{II} molecules were localised inside intact or broken endocytotic vesicles and free in the cell cytoplasm (Fig. 1*d*).

To determine the sequence of Fer-RCA_{II}-receptor interaction and endocytosis, pulse labelling experiments were performed. Cells were labelled with Fer-RCA_{II} for 5–10 min at 0°–4° C, washed by centrifugation, and resuspended in DMEM at 37° C for various times. To duplicate samples, D-galactose was added immediately after the Fer-RCA_{II} pulse at 0°–4° C, or after 5, 15, 30 and 60 min subsequent incubation at 37° C. After the 5 or 10 min pulse of Fer-RCA_{II} at 0°–4° C, the ferritin-lectin molecules located at the cell periphery could be completely or almost completely removed from the cell surface by addition of D-galactose (0.05 M final). After shifting Fer-RCA_{II}-labelled cells to 37° C following labelling and then washing at 0°–4° C, progressively less Fer-RCA_{II} could be removed from cells with time by addition of D-galactose. Also, the location of Fer-RCA_{II} changed from exclusively extracellular in a dispersed distribution (0–5 min after shifting to 37° C), to both extracellular and intracellular (clustered at the

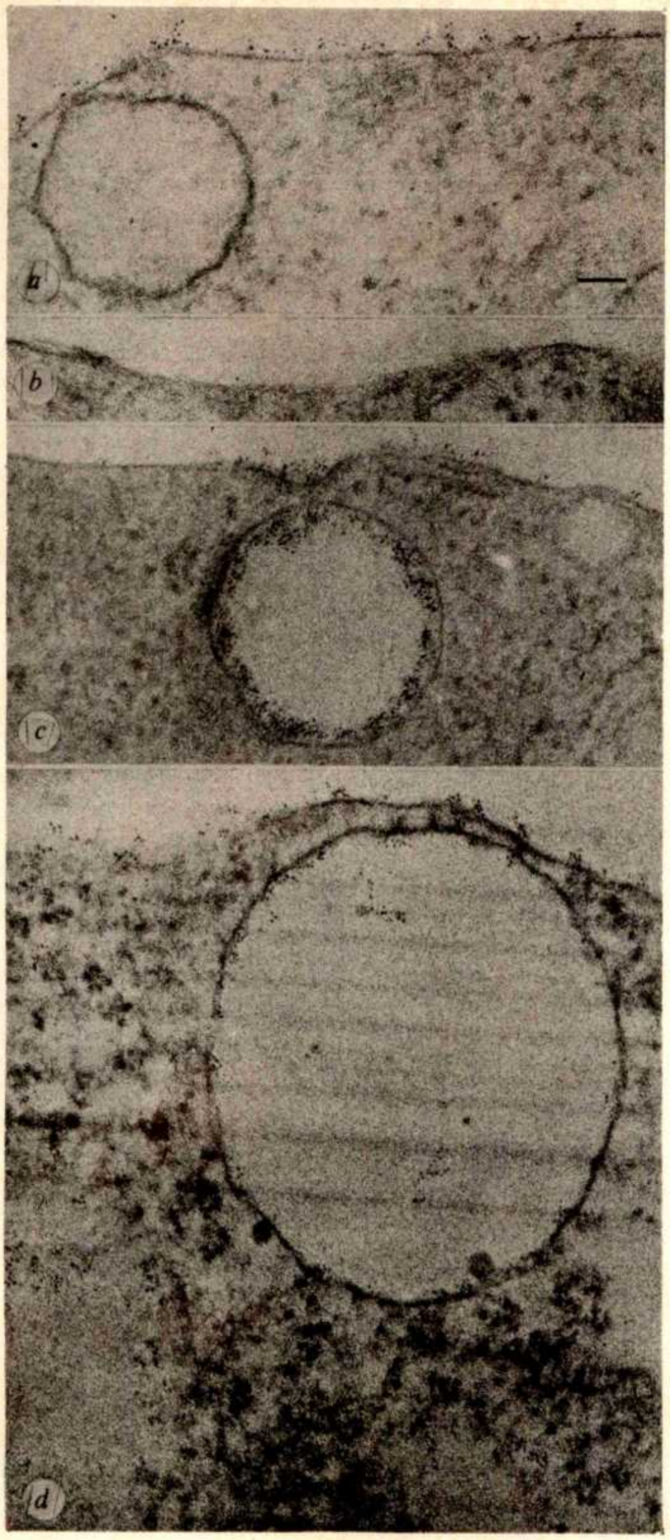

Fig. 1 Fer-RCA$_{II}$ binding to murine 3T3 cells at 37° C. *a*, After a 10 min incubation, ferritin was localised exclusively at the cell surface more or less randomly distributed. Note that the vesicle to the left is unlabelled. *b*, Labelling is specific as D-galactose abolished Fer-RCA$_{II}$ binding activity. *c*, Between 30 and 60 min of incubation Fer-RCA$_{II}$ clustered at the cell surface and endocytosis has begun. *d*, After 60–90 min incubation ferritin was localised inside the cell in intact or broken endocytotic vesicles and free in the cell cytoplasm. All magnifications 68,000; bars equal 0.1 μm.

cell surface and also inside endocytotic vesicles) 30–60 min after shifting to 37° C, to predominantly intracellular (inside endocytotic vesicles and free in the cell cytoplasm) at times greater than 60 min after shifting to 37° C. Generally, the

Table 1 Inhibition of cell protein synthesis by ferritin-conjugated *Ricinus communis II agglutinin*

Pretreatment*		
Additions	Time (min)	Incorporation of ^{3}H-Leu into protein c.p.m.†
None	0	111,700
‡RCA$_{II}$	0	102,100
	10	103,200
	60	2,400
‡RCA$_{II}$ + §D-galactose	60	91,400
¶Fer-RCA$_{II}$	0	105,100
	10	103,300
	60	5,100
¶Fer-RCA$_{II}$ + §D-galactose	60	85,700

* Confluently grown 3T3 cells were removed from substrate with 0.02% EDTA in TC-PBS and washed twice in DMEM containing 5% calf serum. The cells were adjusted to a concentration of 5×10^{6} per ml and 0.5 ml aliquots were incubated with or without Fer-RCA$_{II}$ (~100 μg ml^{-1} total protein) for various times in DMEM at 37° C in a CO$_2$ incubator.

† Cells were treated with Fer-RCA$_{II}$ and washed in DMEM minus leucine and incubated in DMEM plus ^{3}H-leucine (3 μC ml^{-1}) for 60 min at 37° C in a CO$_2$ incubator. The cells were washed three times in TC-PBS and dissolved in 1 ml of 1 M sodium hydroxide containing 0.1% bovine serum albumin. Protein was precipitated by addition of 4–5 ml cold 10% trichloroacetic acid. After a 60 min incubation at 4° C, the contents of each tube were filtered through Whatman G F/C glass filters with vacuum. The filters were washed twice with 5% trichloroacetic acid and once with 95% ethanol and dried. The dried filters were placed in scintillation fluid and counted using a Beckman Model LS200 scintillation counter.

‡ Final concentration, 1 μg ml^{-1}

§ Final concentration, 0.05 M

¶ Final concentration (total protein), approximately 100 μg ml^{-1}

Fer-RCA$_{II}$ that had moved into cells by endocytosis was not susceptible to removal by addition of D-galactose.

Results consistent with these have been obtained with ^{125}I-RCA$_{II}$ (ref. 21). After a 10 min pulse (1 μg ml^{-1} ^{125}I-RCA$_{II}$) at 4° C, approximately 95% of the cell-bound ^{125}I-RCA$_{II}$ could be removed by lactose, but less than 20% could be removed after a 60 min subsequent incubation at 37° C (ref. 21). In parallel experiments less than 5% inhibition of protein synthesis was obtained after the 10 min pulse of RCA$_{II}$ at 0°–4° C. By 30 min after shifting to 37° C, inhibition was approximately 10–20%, and by 60–90 min, it was >80%, similar to the results obtained with ferritin-RCA$_{II}$ (Table 1). In cell-free protein synthesis systems inhibition was almost complete within 1–2 min under these conditions[21]. These studies also stress the importance of performing quantitative lectin labelling experiments for short times at low temperatures to preclude endocytosis of the surface bound lectin molecules[14-16,27,28].

Refsnes *et al.*[22] proposed a two-step model for the toxic action of abrin[15,22,29] and ricin on mammalian cells: in the first step the toxin binds to the cell surface, and in the second step it (or portion of it) is transported into the cytoplasm. My results are consistent with this model, and further suggest that lectin-induced surface aggregation[30-33] and endocytosis[20,21,22] are important in the entry of toxin into mammalian cells. The ultrastructural fate of Fer-RCA$_{II}$ after endocytosis suggests that the lectin molecules are released from endocytotic vesicles, perhaps by breakdown. Lysosomes do not appear to be involved in this process, because I did not observe fusion of lysosomes with the endocytotic vesicles; however, this cannot be ruled out for all vesicles. The fact that simple RCA$_{II}$ binding to cells is not sufficient to cause inhibition of protein synthesis suggests that the endocytosis and release into the cell cytoplasm of RCA$_{II}$ where it can directly interact with polyribosomes is the natural mode of action of this toxin. Other toxins such as diphtheria toxin may enter the cell to act in similar fashion[34,35].

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Serum dopamine β-hydroxylase activity in developing hypertensive rats

SPONTANEOUSLY hypertensive (SH) rats[1] have increased[2,3] tyrosine hydroxylase and dopamine β-hydroxylase (DBH) activities in the adrenal glands. They also show a pronounced hypotensive reaction[2,3] to the specific microbial inhibitors of these enzymes[4]. The results suggest some involvement of the adrenergic nervous system in the hypertension. But the activity of DBH in serum of SH rats, which is released from the sympathetically innervated organs and adrenals[5], does not differ significantly from that of normotensive Wistar rats[6].

We have now compared the developmental change in serum DBH activity in SH rats with that in normotensive Wistar–Kyoto rats. It was from rats of this strain that SH rats were bred, so we felt that they would be better controls than the previously used normotensive Wistar rats. We found that the serum DBH activity in young rats was higher in the SH than in the Wistar–Kyoto strain. We also examined the effect of NaCl administration on serum DBH activities. We found that in 10-week-old rats it caused the enzyme activity to increase simultaneously with a rapid increase in blood pressure, indicating activation of the sympathetic nerves.

SH rats and Wistar–Kyoto rats of the same age were raised in the same conditions. Blood pressure was measured in unanaesthetised animals using the indirect plethysmograph[1]. Blood samples were obtained from rats by decapitating and exsanguinating them into a test tube kept in ice, and serum was removed after centrifuging at 10,000*g* for 10 min. The enzyme activity was assayed by sensitive dual-wavelength spectrophotometry using tyramine as substrate[7]. This method permits the assay of the maximum velocity at a saturated substrate concentration (20 mM) under the optimum *p*H (5.0). The incubation mixture (total volume 1.00 ml) contained: 100 μl of serum diluted to 400 μl with water, 200 μl of 1 M sodium acetate buffer, *p*H 5.0, 100 μl of 0.1 M N-ethylmaleimide, 50 μl of 20 μM $CuSO_4$, 50 μl of 0.2 M sodium fumarate, 50 μl of 0.2 M ascorbic acid, 50 μl of 20 mM pargyline, 1,500 units of catalase in 50 μl of aqueous solution (1 mg ml^{-1}), and 50 μl of 0.4 M tyramine. A sample of boiled enzyme preparation was used as control. Incubation was carried out for 45 min at 37° C. The product, octopamine, was isolated by a microcolumn (0.5 × 10 cm) of Dowex-50-H^+ (packed volume, 0.2 ml), and converted by $NaIO_4$ (10μl of 2% aqueous solution) to *p*-hydroxybenzaldehyde which was then isolated by successive extractions with ether and ammonia, and finally measured by a dual-wavelength spectrophotometer at 333 nm and 360 nm. Octopamine (2.00 nmol) was added to a control incubation as internal standard. The activity was expressed as nmol octopamine formed per min per ml serum.

Table 1 shows that serum DBH activity of both SH and Wistar–Kyoto rats decreased markedly with age after 6 week. This contrasts with the situation in which humans DBH activity increases with age, especially after an erect posture is assumed[8]. The decrease in serum DBH activity could not result from formation of endogenous inhibitors in serum during development, since recovery of pure DBH from bovine adrenal glands added to a rat serum sample was similar (95%) from rats of various ages. One possibility is that the rate of development of the sympathetic nerves in rats may be slower than the increase of blood volume, so that the DBH activity in a volume of serum may decrease with age. The difference between humans and rats in this respect may have the same explanation as the observation that human serum contains a much higher enzyme activity than sera from various animals[9].

The enzyme activity in SH rats after 6 weeks of age is not significantly different from those in Wistar–Kyoto rats. This agrees with our previous result on serum DBH activities of SH rats and normotensive Wistar rats at 16 weeks of age[6]. But at 3 weeks of age, serum DBH activity of SH rats was about two times higher than that of Wistar–Kyoto rats, indicating that the sympathetic nerve activity in SH rats may be increased only when SH rats are very young. The loss of difference between serum DBH activities of SH rats and Wistar–Kyoto rats during development may

Table 1 Serum dopamine β-hydroxylase activities of developing spontaneously hypertensive (SH) rats

Age (weeks)	DBH activity (nmol min^{-1} ml^{-1} serum ± s.e.m.) Wistar–Kyoto rats	SH rats
3	1.18 ± 0.03 (5)	2.23 ± 0.09* (4)
6	0.44 ± 0.02 (4)	0.33 ± 0.01 (4)
12	—	0.22 ± 0.01 (4)
14	0.19 ± 0.04 (6)	0.19 ± 0.03 (6)
	—	0.30 ± 0.07† (10)
16	—	0.17 ± 0.03 (4)
53	0.20 ± 0.02 (4)	0.19 ± 0.03 (4)

*Differs from control (Wistar–Kyoto rats), $P<0.01$.

†NaCl was administered from 10 weeks of age for 4 weeks. Differs from control (SH rats without NaCl administration), $P<0.05$. Numbers in parentheses refer to no. of rats.

be due to a secondary compensatory mechanism in the course of the development of hypertension.

In order to determine the reactivity of the sympathetic nervous system of SH rats, we administered NaCl by supplying 1% NaCl-aqueous solution for drinking instead of tap water after 10 weeks of age for 4 weeks. The blood pressure of control SH rats without NaCl administration was increased from 170 ± 3 mm Hg (at 10 weeks) to 189 ± 3 mm Hg (at 14 weeks), whereas that of SH rats with NaCl administration increased from 173 ± 2 mm Hg (at 10 weeks) to 204 ± 5 mm Hg (at 14 weeks). Thus NaCl administration to SH rats increased blood pressure more pronouncedly. After NaCl administration serum DBH activities were also increased significantly as shown in Table 1. The results suggest that the sympathetic nerve activity in SH rats may be increased only when they are very young and decreased by a compensatory mechanism during the development of hypertension, but may be easily activated if blood pressure is raised acutely.

The elevated serum DBH levels when SH rats are very young and the rapid increase in serum DBH activity during the rapid increase of blood pressure by NaCl administration suggest some role for the sympathetic nervous system in the onset and maintenance of hypertension in SH rats.

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Enzymatic synthesis of acetylcholine by a serotonin-containing neurone from *Helix*

ACETYLCHOLINE (ACh) and serotonin are strong neurotransmitter candidates in the snail species *Helix aspersa* and *Helix pomatia*[1,2]. Experiments[1] have indicated that the enzymes responsible for their biosynthesis, choline acetyltransferase (ChAc, EC 2.3.1.6.) and L-aromatic amino acid decarboxylase (AAD, EC 4.1.1.26.), were specifically localised to separate populations of neurones in *H. aspersa*, and similarly for *H. pomatia* (M.R.H., unpublished). Out of 150 neurones, 36 were found to contain ChAc only (detection limit 2 pmol h^{-1}) and 20 were found to contain AAD only (detection limit 8 pmol h^{-1}). This clear biochemical differentiation between presumably 'cholinergic' neurones and presumably 'serotonergic' neurones has been previously noted in other invertebrates[3,4]. It was therefore surprising when, in two laboratories (St Andrews and Kjeller), we independently found that the two homologous giant metacerebral neurones, which contain serotonin[5] and are thought to use it as a neurotransmitter[6], could also enzymatically synthesise ACh. The coincidence of these two enzymatic activities in the giant serotonin cell (GSC) is a unique and therefore intriguing situation among characterised invertebrate nerve cells.

In experiments at Kjeller, ChAc was assayed as described by Fonnum[7] with modifications in the incubation medium to yield optimal activity for *Helix*[8]. In experiments at St Andrews, ChAc was assayed by the same method as modified by Coggeshall *et al.*[9] using the following incubation medium (final concentration): 200 μM ^{14}C-acetyl-CoA, 20 mM choline chloride, 300 mM NaCl, 1 mM EDTA. $2H_2O$, 80 mM sodium phosphate buffer (*p*H 7.4), 0.1 mM eserine, and 0.5% (v/v) Triton X-100. The cells were incubated for 1 h at 37° C (to give maximal *in vitro* activity), and the blanks were 1.5–2.0 pmol. In one series of experiments, both ChAc and AAD were assayed in the same incubation, using the following medium: 0.45 mM ^{3}H-acetyl-CoA, 0.2 mM D,L-1-^{14}C-DOPA, 8 mM choline bromide, 250 mM NaCl, 20 mM sodium phosphate

Table 1 ChAc activity of the giant metacerebral cells (GSCs) after different treatments

Treatment	Activity *H. aspersa*	(pmol $cell^{-1}$ h^{-1}) *H. pomatia*
Control	22.3±6.3(4)	20.6±2.0(8)
Incubation medium + AChE-eserine	<2 (3)	<2 (3)
Incubation medium—choline	<2 (3)	<2 (3)
Incubation medium + Acryloylcholine (1.25 mM)	<2 (3)	<2 (5)
Incubation medium + Chloroacetylcholine (0.125 mM)	<2 (3)	<2 (5)
Incubation medium + CoA (100 mM)	10.8±3.7(3)	10.4±2.2(3)
Incubation medium + ACh (20 mM)	13.0±2.9(3)	11.4±2.5(3)
Incubation medium + $MgCl_2$ (100 mM)	8.9±4.2(3)	11.2±2.6(3)
Incubation medium + $CaCl_2$ (100 mM)	3.6±1.7(3)	5.5±2.3(3)
Heat denaturation (1 h at 90° C)	<2 (3)	<2 (7)
TCA precipitation	<2 (3)	<2 (7)

Results are expressed as means ± s.e.m. The numbers in parentheses refer to no. of values used for mean.

buffer (*p*H 7.4), 0.1 mM eserine, 0.1 mM pyridoxal phosphate, 0.125 mM nialamide, 11.5 mM EDTA, and 0.2% (v/v) Triton X-100. The assay was based on the absorption in hyamine of the evolved ^{14}C-CO$_2$ from DOPA, and the extraction of ^{3}H-ACh by Kalignost as before[7]. Animals were maintained in artificial hibernation by storage in a humid environment at 10° C. The dissection was performed as described elsewhere[1]. In some experiments, the removal of single cells was expedited by conducting the dissection under 70% ethylene glycol (−20° C). Enzyme activities were found to be unaffected by the choice of dissection solution, as noted earlier[4]. All experiments were conducted in late spring and summer.

The identification of the extractable radioactivity as acetylcholine was based on the following observations (see Table 1). First, electrophoresis of the extract gave a single radioactive product comigrating with standard ACh (Fig. 1) in two sets of conditions[4,10]; second, no activity was found in the absence of choline; third, no activity was found in the presence of acetylcholinesterase and the absence of cholinesterase inhibitor and, fourth, Kalignost extraction completely separated ACh from acetylcarnitine and other possible acetylated contaminants[11].

Synthesis of ACh was abolished on boiling the cells or after trichloroacetic acid (TCA) precipitation (Table 1), indicating that it is enzymatically mediated. The enzymatic ACh synthesis was inhibited by the presence of MgCl$_2$, CaCl$_2$, ACh, CoA, acryloylcholine, and chloroacetylcholine, in concentrations known to similarly affect the partially purified ChAc from whole snail brain[8]. The latter two substances should be regarded as fairly specific ChAc inhibitors, and inhibited the GSC enzyme to the same degree as they did other choline acetyltransferase-containing neurones.

The level of ACh synthesis by the GSC was highly reproducible in this assay. It was lower than that of a neurone found on the posterio-dorsal surface of the visceral ganglion (known as cell no. 21 (ref. 1) on cell no. 4 (ref. 12)), but comparable to that of the medial buccal cell (cell no. 4 (ref. 1)), which was considered to have a relatively low ChAc activity. Other cells known to contain large amounts of serotonin or AAD, such as the one localised near the right parietal ganglion in the posterio-dorsal surface of the visceral ganglion (known as cell no. 11 (ref. 1) on cell no. 4 (ref. 13)), contained insignificant ChAc activity. These cells served as controls that the observed ACh synthesis in the GSC was not in any way associated with serotonin-metabolising enzymes. Using the double assay, the uniqueness of the GSC was reaffirmed as both enzymes could indeed be measured simultaneously, whereas in control cells, either one activity or none was observed (Table 2). A control extract of 0.5 µl dissecting medium taken from the area of the GSC dissection did not reveal any background activity liberated by tissue disruption (Table 2).

Examination of related species having GSCs[5] revealed that they also share this dual-enzyme characteristic in these neurones, albeit the activity varies somewhat (M.R.H., G.A.C., P.C.E., and F.F., unpublished).

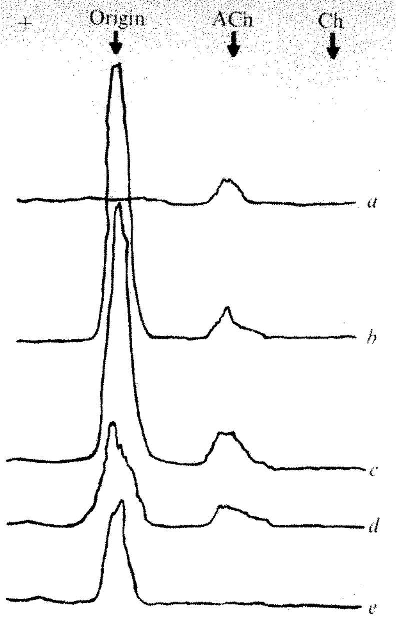

Fig. 1 Electrophoresis of the choline acetyltransferase assay product. Samples were electrophoresed for 50 min in the conditions of Potter and Murphy[9], and subsequently scanned for radioactivity in a Nuclear Chicago Actigraph III (960 V high voltage, 1 s counting, chart speed of 240 cm h^{-1}, 6 mm collimator slit width). Scans *a* and *b* represent the results of assays using a 0.8 µl extract of snail brain homogenate as the source of enzyme. In *a*, 50 µl of Kalignost extraction of the completed incubation was applied. In *b*, 2 µl of the unextracted, completed incubation was applied. In *c*, six GSCs were used as the enzyme source, and in *d*, one cell 21 neurone. Scan *e* is the control to which no enzyme was added. Acetylcholine (ACh) and choline (Ch) mobilities were determined by staining added standards with iodine vapour.

There is consequently strong evidence that acetylcholine can be synthesised in a known serotonin-containing neurone by a choline acetyltransferase.

Assuming that the ChAc serves a physiological role of ACh production in the GSC, it is worthwhile considering the functions of ACh and serotonin within the neurone. Both may serve as neurotransmitters, in which case the long-held dogma of each neurone making and utilising a single neurotransmitter must be abandoned[14]. In this regard, it should be noted that the GSC ChAc is more active than the base levels we quote here during those months when it is difficult to elicit responses to serotonin from the follower cells[6]. Far more likely is the possibility that either the ACh or serotonin (or both) serves as alternative cellular or hormonal function besides classical neurotransmission, perhaps modulating metabolic activity of innervated regions. Last, it should be considered that the ChAc activity might arise from contamination, either by invaginating glia or adhering microneurones. This can never be excluded when work-

Table 2 Enzyme activities of single neurones using both single and double assays for ChAc and AAD in *Helix aspersa*

Neurone	Location	ChAc activity pmol cell^{-1} h^{-1}	Double assay	
			ChAc activity pmol cell^{-1} h^{-1}	AAD activity
GSC	Ventral surface of metacerebral ganglion, near input of inner lip nerve	22.3±6.3(4)*	19.8±9.0(6)	26.0±14.3(6)
Medial buccal cell	Lateral edge of dorsal surface of buccal ganglia. Medial of group of three giant neurones	21.0±5.0(4)	20.3±10.2(3)	<8 (3)
Cell 21	Posterio-dorsal surface of visceral ganglion, between inputs of cutaneous pallial nerve and visceral nerve	65.6±7.9(5)	56.3±12.0(3)	<8 (3)
Cell 11	Posterio-dorsal surface of visceral ganglion, bordering right parietal ganglion	<2 (5)	<2 (3)	56.4±7.5(3)
Posterior buccal cell	As medial buccal cell. Posterior of group of three giant neurones	<2 (5)	<2 (3)	<8 (3)
	Control extract from area of GSC dissection (0.5 µl)	<2 (8)		

* Numbers in parentheses refer to number of determinations used to derive figure.

ing with hand-dissected preparations[15]; but the specific localisation of the enzymes to particular neurones and the absence of ChAc activity from the dissection area do not support this objection.

The possibility that the ACh in the conditions of the assay, is synthesised by an enzyme other than ChAc cannot be excluded. It seems very unlikely, however, in view of the effect of the ChAc inhibitors. Carnitine acetyltransferase (EC 2.3.1.7.) and aryl amine N-acetyltransferase (EC 2.3.1.5.) have been discounted on the basis of their hundredfold lower activities and different kinetic parameters (P.C.E., and M.R.H., unpublished).

Further work will focus on the direct measure of ACh and its functional role in the GSC.

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Assignment of the gene for galactokinase to human chromosome 17 and its regional localisation to band q21-22

PROGRESS in somatic cell genetics now allows more rapid and precise localisation of genes within the human genome. A large number of mouse × human hybrid cell lines are available, permitting the investigator to choose, for phenotype assay, lines with a particular reduced human chromosome complement. An increasingly large number of hybrid lines carrying rearranged chromosomes are also becoming available for regional localisation studies. We have used such hybrid cell lines to assign a gene coding for galactokinase (EC 2.7.1.6) to human chromosome 17 and to further localise the gene to band 21–22 on the long arm of the chromosome. Results from this study have provided new information regarding the functioning of the genes coding for the Leloir pathway enzymes in man[1], and have revealed a relatively close and potentially useful linkage between the genes for galactokinase and for thymidine kinase (EC 2.7.1.21).

The 70 mouse × human cell lines used in this analysis were produced, selected and propagated by methods reviewed elsewhere[2]. Hybrid series 41 is composed of 14 primary cell lines, 11 of known independent origin, isolated after fusion of mouse temperature sensitive line ts-1 (ref. 3) with GM 126, human skin fibroblasts. Series 50c consists of 5 primary lines, 4 of known independent origin, isolated after fusion of mouse line TA3 (ref. 4) with human peripheral blood leukocytes. The AIM series consists of 11 primary lines, 9 of known independent origin, isolated after fusion of mouse line A9 (ref. 5) with human skin fibroblasts. The YA series consists of 20 primary lines, 8 of known independent origin, isolated after fusion of A9 with human skin fibroblasts.

We also used a panel of unrelated hybrid lines chosen from a collection of mouse × human hybrids on the basis of their specific partial human karyotypes. Only a few different human chromosomes are represented within a given hybrid line, the same chromosomes being present in many or most of the individual cells. The specific human chromosomes represented varied widely between hybrids of the panel. Analysis of this set of cell lines, then, generally allows the rapid and unequivocal correlation of a given human phenotype with a particular human chromosome. Hybrids which composed the panel were five subclones of JFA-14a and six of JFA-16a (two independent hybrid lines resulting from a fusion of A9 with human skin fibroblasts[6]), WA-Ia-D6[6] (A9 × WI-38[7], human embryonic lung fibroblasts) and IL-II-5[8] (mouse L cell line LM(TK$^-$)[9] × IMR 32, human neuroblastoma).

For regional localisation studies we used two hybrid lines from the WL series[10], WL-24a-2-A and WL-24a-5-C, plus five subclones of WL-24a-2-A isolated after treatment of the hybrid line with adenovirus-12 (ref. 11). WL hybrids originated from a fusion of LM(TK$^-$) with WI-38. They were maintained in HAT selective medium[12] in which cells retaining the human thymidine kinase gene complementing the mouse deficiency survive. Neither of these two hybrids, however, retained an intact human chromosome 17, the chromosome to which the cytosol form of thymidine kinase has been assigned[13-15]. In WL-24a-5-C a portion of the long arm of 17 persists as a marker chromosome, t(11; 17) (q22; q21), which arose

Table 1 Enzymatic activities assayed in hybrids and their respective human chromosome assignments

Chromosome	Enzyme	
1	Pep C	peptidase C (EC 3.4.3.–)
2	IDH-1	isocitrate dehydrogenase (EC 1.1.1.42)
2	MDH-1	(NAD)-malate dehydrogenase (EC 1.1.1.37)
6	ME-1	(NADP)-malic enzyme (EC 1.1.1.40)
7	MPI	mannose phosphate isomerase (EC 5.3.1.8)
10	GOT	glutamate oxaloacetate transaminase (EC 2.6.1.1)
11	LDH A	lactate dehydrogenase (EC 1.1.1.27)
12	Pep B	peptidase B (EC 3.4.3.–)
14	NP	purine nucleoside phosphorylase (EC 2.4.2.1)
16	APRT	adenine phosphoribosyltransferase (EC 2.4.2.7)
18	Pep A	peptidase A (EC 3.4.3.–)
19	GPI	glucose phosphate isomerase (EC 5.3.1.9)
20	ADA	adenosine deaminase (EC 3.5.4.2)
21	SOD-1	cytoplasmic indophenol oxidase or superoxide dismutase-1
X	G6PD	glucose-6-phosphate dehydrogenase (EC 1.1.1.49)
X	PGK	phosphoglycerate kinase (EC 2.7.2.3)

Table 2 Number of hybrids showing presence (+) or absence (−) of the two given human enzymatic activities

	+/+	+/−	−/+	−/−	Total
GaK/IDH-1	5	18	3	24	50
GaK/MDH-1	6	17	7	20	50
GaK/Pep A	17	6	10	17	50
GaK/TK	16	2	0	20	38

Hybrids assayed for IDH, MDH-1 and Pep A are from series 41, 50c, AIM and YA. Hybrids assayed for TK are from series AIM, YA and WL.

spontaneously in the hybrid[16]. In WL-24a-2-A the entire long arm of 17 has been translocated to a mouse chromosome, (M; 17q). In both cases, the translocated product represents the sole identifiable human chromosome element in the hybrid. Treatment of WL-24a-2-A with adenovirus-12 induced breaks in the translocation chromosome (M; 17q) specifically in the region 17q21-22. In subclones A12/1B and A12/1D, the break occurred in 17q21. In subclones A12/2A, A12/2C, and A12/2D, a break occurred in 17q22. Terminal deletions occurred distal to these breaks. These lines have been used previously to assign the gene coding for thymidine kinase to human chromosome band 17q21-22 (ref. 11).

Extracts of the hybrid lines were assayed by vertical starch gel electrophoresis for the presence of human electrophoretic forms of 16 enzymes. Genes coding for these enzymes have previously been mapped to specific human chromosomes, thus the presence of the human isozyme is considered to be strong evidence for the presence of the corresponding human chromosome in the hybrid line. The enzymes used as markers in this study are listed in Table 1 with their chromosome assignments. Methods of assay have been described elsewhere[17]. Thymidine kinase activity was assayed on Cellogel (Chemetron, Milan, Italy) using the method of Migeon *et al.*[18]. Electrophoresis was carried out at 4° C for 4 h at 30 V cm^{-1} using a buffer of 50mM TES (N-*tris*(hydroxy-methyl)methyl-2-aminoethane sulphonic acid) adjusted to *p*H 6.2 with triethanolamine. Our method used to assay galactokinase activity on vertical starch gels has been described[19], (Fig. 1).

Enzymatic analysis of the hybrids from series 41, 50c, AIM and YA indicated that galactokinase (GaK) activity segregated discordinately with the 16 enzymes used as chromosome markers. A representative data sample given in Table 2 shows discordant segregation of GaK with IDH-1 and MDH-1, two markers on chromosome 2 and with Pep A, a marker for chromosome 18. These two chromosomes plus 12 others corresponding to the remaining 13 enzymes could thus be eliminated from consideration in attempts to assign the gene coding for GaK activity to a particular human chromosome. When the panel of well-karyotyped hybrid lines were assayed or GaK activity (Table 3) and conversely the 11 AIM hybrids were karyotyped this conclusion was further substantiated. The 14 chromosomes which were eliminated from consideration on the basis of the enzyme assays could also be eliminated on the basis of chromosome analysis of the hybrids. Nine additional chromosomes which either carried no easily assayed enzyme marker or carried markers which were not included in our assays could also be eliminated from consideration on the basis of chromosome analysis. It then became clear that only chromosome 17 segregated concordantly without exception with GaK activity. The gene for GaK was assigned on this basis to human chromosome 17. The assignment was reinforced by thymidine kinase (TK) assay of the AIM, YA, and WL hybrids. With only two exceptions where human GaK appeared to be present in a hybrid and TK not, the two enzymatic activities segregated concordantly (Table 2).

More precise localisation of the GaK gene along the length of chromosome 17 was made possible by analysis of the WL hybrids. Cell line WL-24a-2-A carrying the (M; 17q) translocation chromosome retained human GaK activity restricting the map position of the gene to the long arm of the chromosome. Hybrid WL-24a-5-C which carried the t(11; 17)(q22; q21) marker likewise showed human GaK activity further restricting the gene to a position in or distal to 17q21. WL-24a-2-A subclones A12/1B and A12/1D which had lost the chromosome portion distal to a break in 17q21 had also lost GaK activity. Subclones A12/2A, A12/2C and A12/2D in which the chromosome loss involved only regions distal to the break in 17q22 retained GaK activity. The GaK gene, from this evidence, must be located between the two breakpoints, and we have accordingly assigned the gene to human chromosome 17 band q21-22 (Fig. 2).

Analysis, then, of the chromosome and enzyme complement of a large number of independent hybrid cell lines of diverse mouse × human parentage has yielded compelling evidence for the assignment of the galactokinase gene to human chromosome 17. GaK activity segregated discordantly with 16 marker enzymes and concordantly with only chromosome 17 and TK activity. Data from two WL subclones which were functionally back-selected for loss of TK activity indicate that GaK activity is lost concordantly with the loss of a portion of chromosome 17. Information obtained from the WL hybrids allowed us to further restrict the map position of the galactokinase gene to band q21-22. The discrepant data which we have obtained, that regarding discordant segregation of TK with GaK in two YA hybrids, could be explained either by the difficulty in assaying TK or by a possible breakage and rearrangement of chromosome 17. We have not been able to identify an intact 17 in one of the discrepant hybrids.

Only chromosome 17 is consistently present in hybrid lines which show human GaK activity as substantiated by the chromosome data (Table 3) and the complementary results

Table 3 Human GaK activity and human chromosomes present in hybrid cell lines

Hybrid	Human GAK	Human chromosomes																							
		1	2	3	4	5	6	7	8	9	10	11	12	X	13	*14	15	16	17	18	19	20	21	*22	Y
JFA-16a-5	—		+			+			+			+				+		+		+		+		+	
JFA-16a-8	+		+			+	+		+			+				+			+	+		+		+	
JFA-16a-15b	—		+			+						+								+		+			
JFA-16a-19	—		+			+						+				+				+		+		+	
JFA-16a-21	—		+									+								+		+			
JFA-14a-5	+																		+						
JFA-14a-10c	+	+	+			+			+						+			+	+	+					
JFA-14a-12	+		+												+				+	+					
JFA-14a-13	+		+			+												+	+	+					
JFA-14a-23b	+		+															+	+	+					
JFA-14a-6b	+		+															+	+	+					
WA-Ia-D6	—	+	+		+					+	+	+	+	+	+			+	+	+		+	+		
IL-II-5	+								+				+						+		+				

*t(14;22).

Karyotypes of hybrids were analysed using air dried preparations[24] stained with quinacrine mustard or Atebrin to produce fluorescent banding of the chromosomes[25] and stained subsequently with Giemsa to reveal constitutive heterochromatin[26]. At least 20 cells from each hybrid line were analysed for the presence of human chromosomes. In all cases specific chromosomes were scored as present in the hybrid if they were found in at least 10% of the cells examined.

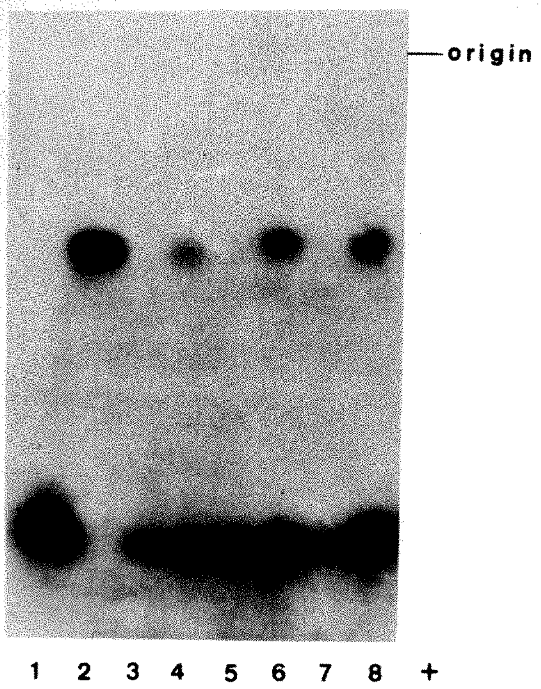

Fig. 1 Galactokinase assay of five WL-24a-2-A subclones. After electrophoresis in citrate phosphate buffer at *p*H 6.8, the gel was incubated in the reaction mixture of Mayes and Guthrie[27]. ^{14}C-galactose-1-phosphate produced by the enzyme was then precipitated in the gel with lanthanum chloride and subsequently detected by autoradiography[28]. Omission of ATP from the reaction mixture prevented the appearance of the reaction spots. Addition of 10 mM D-glucose did not. Mouse control, LM(TK$^-$), was run in channel 1. Human control, KB, is shown in channel 2. Channels 4, 6 and 8 represent extracts of subclones A12/2C, A12/2A and A12/2D, respectively, all of which show both mouse and human forms of the enzyme. Channels 3 and 7 represent extracts of A12/1B and channel 5 represents A12/1D, neither of which shows the human activity band.

from enzyme analysis (Table 2). In addition, hybrid WL-24a-2-A which shows human GaK activity carries the long arm of 17 as its sole human chromosomal element. For this reason we feel that the genetic locus which we have mapped codes for a structural element of the enzyme. In this respect it might be noted that galactokinase purified from human red blood cells is reported to consist of two subunits of approximately equal size with only the dimeric form showing enzymatic activity[20]. We find only one spot of activity for human galactokinase by our assay method and might suppose that it represents this dimer. It is surprising then that no heteropolymer of intermediate electrophoretic mobility forms between human and mouse isozymes in the hybrids as has been observed with numerous other multimeric enzymes[17].

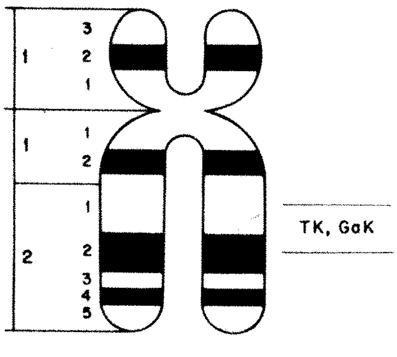

Fig. 2 Chromosome 17 as given by the Paris Conference (1971) (ref. 29) indicating the assigned map position for GaK.

Galactokinase is the first of three enzymes of the Leloir pathway. Since the structural genes for these enzymes map within the same operon in *Escherichia coli*[21] and are closely linked also in yeast, *Saccharomyces cerevisiae*[22], it is interesting to know whether the corresponding genes are linked in man. Human galactose-1-phosphate uridyltransferase (EC 2.7.7.12) has been assigned in two conflicting reports to chromosome 2 (ref. 23) and 3 (personal communication from T. A. Tedesco), and we assign galactokinase to 17. It seems, then, that the genes coding for these two enzymes of the pathway in man are probably not linked.

Linkage of the gene coding for galactokinase with that for thymidine kinase on the other hand, is one of the closest linkages to be detected using the techniques of somatic cell genetics. Since thymidine kinase is a widely used selective marker, these two genes form an especially useful gene pair. For example, it will be of interest to test the possible coordinate transfer of these markers in gene transfer experiments.

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5-Methylcytosine localised in mammalian constitutive heterochromatin

DEOXY-5-METHYLCYTIDYLIC acid (5-MeC) is an almost universal, although minor component of DNA in plants and animals. It makes up 4–7% of the bases in plants[1], no more than 1.5% of the bases in the mouse[2] and even less in the human[3]. 5-MeC itself is not incorporated directly into DNA. Instead, specific deoxycytidylic acid residues in preformed DNA are methylated enzymatically by a highly specific DNA methylase, which transfers a methyl group from S-adenosyl methionine to specific sites in DNA[4]. In the mouse, as in other mammals, virtually all of the 5-MeC is in the 5-MeCpG doublet[5], which is distributed nonrandomly in the DNA. Mouse satellite DNA contains about 3% 5-MeC in comparison to about 1.3% in main band DNA[2] and is located at the centromeric end of nearly every chromosome[6].

Antisera to 5-methylcytosine (anti-5-MeC) are available (ref. 7 and Sawicki, Erlanger and Beiser, unpublished) and these can react with 5-MeC residues in single-stranded DNA. Earlier we showed that antibodies specific for particular purine or pyrimidine bases[8], which react with denatured DNA but not native DNA[9], can bind to fixed metaphase chromosomes. The major sites of binding can be localised by immunofluorescence[10,11]. These antibodies can therefore serve as probes of mammalian chromosome organisation. After ultraviolet irradiation, for example, of fixed mouse or human chromosomes, anti-adenosine (anti-A) and anti-thymidine antibodies bound intensely to the AT-rich constitutive heterochromatin[12].

We have now investigated the chromosomal location of 5-MeC residues in both the mouse and the human. Chromosome preparations which had been either ultraviolet irradiated or heated to 85° C in 0.07 M Sorensen's buffer at *p*H 6.5 for 15 or 30 min and chilled suddenly to prevent reassociation, were exposed to one or other of two different anti-5-MeC antisera. These antisera showed no cross reaction with thymidine or with cytidine after absorption with an excess of cytidine conjugated to rabbit serum albumin. The immunofluorescent banding of chromosomes with these two antisera was thus a result of specific binding to 5-MeC residues in the DNA and could be totally abolished by competition with added 5-MeC.

In the mouse, anti-5-MeC showed preferential binding to the C-band regions of centromeric heterochromatin (Fig. 1). The fluorescent regions were very small on a few of the larger chromosomes. These appeared to be the same chromosomes (1 and 3) which have very little centromeric heterochromatin in the AKR strain of mouse used in these experiments[13]. The intense fluorescence of the C-band regions partly reflects the greater concentration of 5-MeC in mouse satellite DNA than in main band DNA[2], but also the greater degree of ultraviolet-induced strand separation in the very AT-rich satellite DNA, which contains sequences of up to four thymine residues in a row in its light strand, and up to five in its heavy strand[14].

The presence of 5-MeC in mouse satellite DNA indicates that the repeating nucleotide sequence in this DNA contains the 5-MeCpG doublet. The amount of 5-MeC, 3 mol% (ref. 2), sets a lower limit on the length of the repeating sequence in the satellite DNA. Assuming that each strand is methylated equally, and taking into account the presence of a small proportion of non-identical sequences, this repeating sequence must be about 30 nucleotides long or some multiple of this. This fits rather well with the estimates of Hutton and Wetmur[15] and Sutton and McCallum[16]. Sutton's estimate of 8–13 nucleotides[17] is clearly too short unless the repeating sequence contains even shorter repeating sequences, some of them not containing 5-methylcytosine.

In the human, the pattern of anti-5-MeC binding was virtually identical whether strand separation was achieved by ultraviolet irradiation or by heating to 85° C. In both cases anti-5-MeC showed preferential binding to the C-band regions of constitutive heterochromatin of chromosomes 1, 9 and 16, the centromere–short arm region of chromosomes 15, and the distal part of the Y (Fig. 2). The C-band region of chromosomes 10, 17, 22 and Y sometimes showed a small region of enhanced anti-5-MeC binding. The observed pattern of binding, and the absence of comparable binding of anti-5-MeC to the C-band regions of most of the chromosomes suggests that methylation occurs only in certain clases of repetitive DNA. For example, the strongly positive regions in chromosomes 1 and 16 are the chief sites

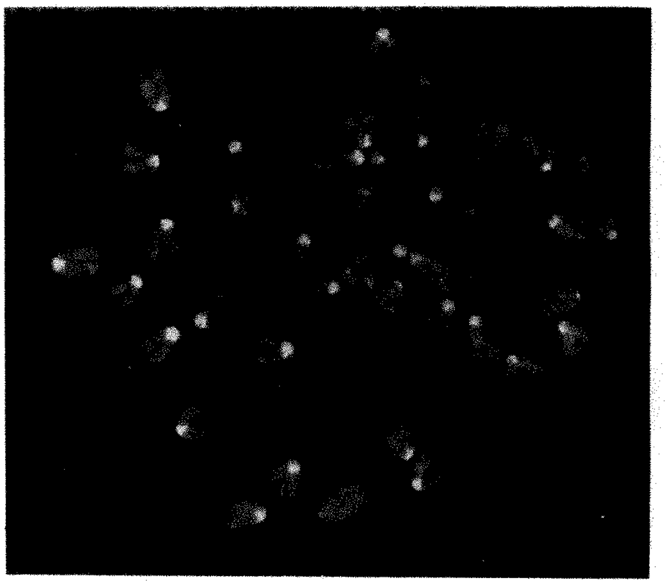

Fig. 1 Metaphase spread from an AKR strain mouse showing sites of anti-5-MeC binding. Note the bright fluorescence at the centromeric end of most chromosomes and the low level of fluorescence of the remainder of all the chromosomes. Mitotic cells from a primary embryonic culture were expanded with 0.075 M KCl hypotonic solution, fixed in three changes of methanol:glacial acetic acid (3:1) and air dried. The spreads were ultraviolet irradiated for 16 h (30 cm from a germicidal lamp) either dry or in Sorenson's buffer, incubated for 30 min with anti-5-MeC at room temperature, washed thoroughly with phosphate buffered saline (PBS: 20 g NaCl, 85 ml 0.25 M Na_2HPO_4, 15 ml 0.25 M KH_2PO_4 in 2,400 ml distilled water H_2O, *p*H 7.2–7.4), incubated for 30 min with fluorescein-tagged anti-rabbit gammaglobulin prepared in sheep, washed thoroughly with PBS, mounted in PBS and analysed by fluorescence microscopy using transmitted light from an HBO 200 W high pressure mercury lamp delivered through a cardioid condenser, BG-12 exciter filter and K530 barrier filter. The photograph was taken on Kodak Plus X film with a 2 min exposure, developed with Acufine and printed on Ilford No. 4 grade paper.

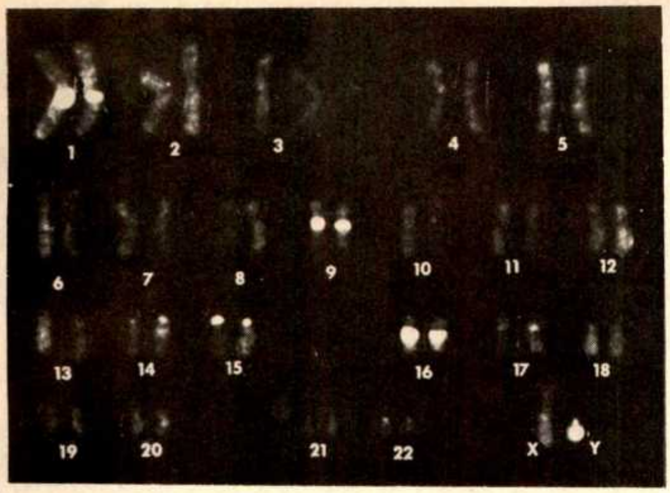

Fig. 2 Karyotype of a human cell showing sites of anti-5-MeC binding. Note the intensely bright fluorescent regions on chromosomes 1, 9, 15, 16 and the Y, and the difference between homologues in each case in the size of this region. Smaller bright centromeric dots are present on one or both copies of chromosomes 14, 17, 22 and Y. Technique as in Fig. 1. Chromosomes were identified by restaining the cell with quinacrine mustard and rephotographing, using the same fluorescence microscopy system and film but with an exposure of only 9 s instead of the 2 min necessary with the antibody.

of human satellite II DNA as revealed by in situ DNA–RNA hybridisation[18], whereas the corresponding 5-MeC-positive region of chromosome 9 contains both satellite II DNA[18] and satellite III DNA[19]. Our results suggest that satellite II, and perhaps satellite III, contain 5-MeC. We are unaware of a direct chemical analysis of the 5-MeC content of the various human satellite DNAs. Since neither satellite II nor satellite III is abundant in chromosome 15 or the Y, these chromosomes must contain different types of highly repetitious, methylated DNA.

The immunofluorescence approach described here can be used to locate classes of highly repetitive DNA which contain 5-MeC. It is already apparent that these classes show remarkable quantitative variation between homologues and among individuals. These differences provide chromosome markers which can be used in genetic linkage studies or in the analysis of structurally altered chromosomes. This might be particularly useful in identifying a structurally altered chromosome 15, since its proximal portion can now be more specifically identified than before. Striking differences have been noted in comparing anti-5-MeC binding to chromosomes of gorilla, chimpanzee and orang utan (unpublished data); and the technique seems to offer a promising approach to the evolution of satellite or other classes of highly repetitive DNA.

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Structure of DNA in DNA replication mutants of yeast

As in higher eukaryotes[1,2], the nuclear DNA of the simple eukaryote *Saccharomyces cerevisiae* is replicated from multiple internal initiation sites along the chromosome[3–5]. The very small size of the chromosomal DNA in yeast[3,6,7] and the availability of temperature-sensitive DNA synthesis mutants[8,9] make a detailed study of DNA replication easier in yeast than in most other eukaryotic systems. In this report, nuclear DNA molecules from several DNA synthesis mutants which had been classified as DNA initiation or DNA propagation mutants on the basis of incorporation kinetics were examined with the electron microscope. Our results provide independent criteria for the classification of DNA initiation and propagation mutants on the basis of the structure of the nuclear DNA at the restrictive temperature.

Three temperature-sensitive DNA replication mutants were used in these studies: *cdc*4, *cdc*7 and *cdc*8 (refs 8,9). If synchronous cultures of cells carrying mutations in *cdc*4 or *cdc*7 are shifted to the restrictive temperature immediately after initiation of DNA synthesis has occurred, approximately a complete round of DNA is made before synthesis stops[8,9]. It is been suggested, therefore, that the gene functions *cdc*4 and *cdc*7 are required for the initiation of nuclear DNA synthesis[8,9]. The gene function *cdc*8 is necessary for DNA propagation since cells carrying this mutation abruptly shut off nuclear DNA synthesis when shifted to the restrictive temperature[8].

When DNA molecules from *cdc*4 at the restrictive temperature were examined in the electron microscope, all molecules (126/126) were simple linear structures, containing neither replication 'bubbles' ('eye-forms') nor Y-shaped replication forks. In DNA isolated from *cdc*7 at the restrictive temperature (36° C), 144/150 molecules were simple linear structures. Six of the molecules contained replication forks, possibly the result of some leakiness of DNA syn-

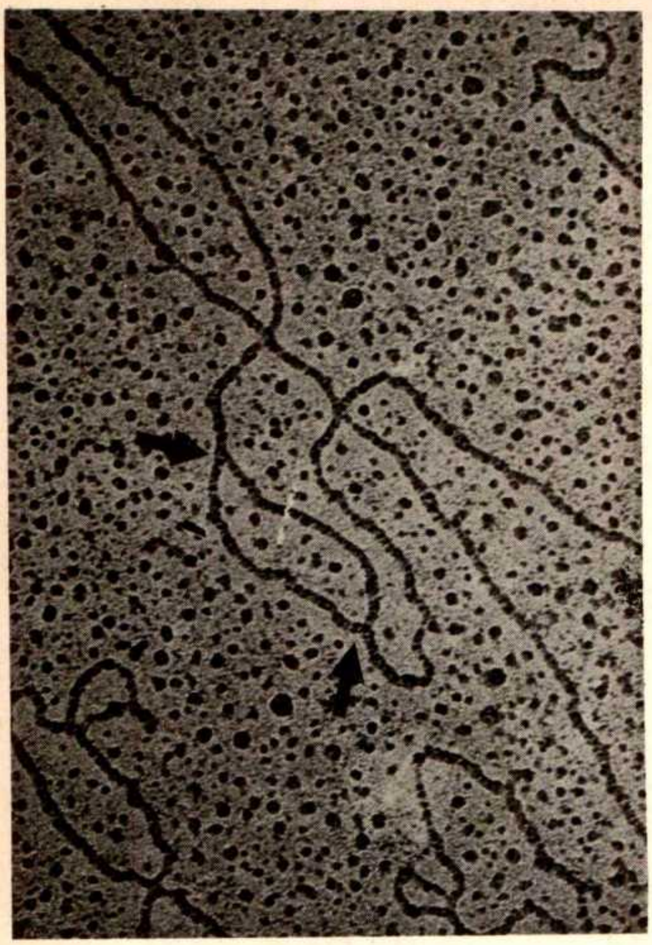

Fig. 1 Small DNA replication bubble in DNA isolated from a temperature-sensitive DNA propagation mutant (*cdc*8) at the restrictive temperature. A culture of *cdc*8 (strain 198–1) was grown at 23° C in 10 ml of Y-minimal media[6] supplemented with 0.2 g glucose, 5 mg yeast extract, 200 μg uracil, 200 μg adenine, 500 μg tyrosine, 500 μg histidine and 500 μg lysine. The doubling time of the culture under these conditions is approximately 3 h. At a cell density of 2×10^6 cells ml^{-1}, the culture was shifted to 36° C for 5 h. Cells were then collected and spheroplasted as described previously[6]. 0.2 M hydroxyurea (Matheson, Coleman and Bell, Norwood, Ohio) was added to the spheroplasting solutions. Nuclear DNA was separated from other cellular components by centrifugation in sucrose gradients[3]. Methods for spreading DNA for electron microscopy have been described[3]. Each branch of the bubble in the photographed molecule is 0.4 μm in length; arrows show the forks. Nuclear DNA was prepared from *cdc*4 (strain 135.1.1.) and *cdc*7 (strain 4008) as described above. For both initiation mutants all the DNA molecules observed in the experiments should come from cells arrested in the cell cycle at the beginning of the S period[8,9]. For *cdc*8, however, although most of the molecules of DNA should come from cells blocked near the beginning of the S period, some DNA molecules should come from cells within the S period. Since only 3–5% of the DNA molecules in an asynchronous culture are replicating at any given time[3], most of the DNA molecules observed in the *cdc*8 experiment should contain no replication structures before the shift to the restrictive temperature.

thesis at the restrictive temperature. This allele of *cdc*7 (4008) is somewhat leaky at 36° (ref. 9) synthesising nuclear DNA at approximately 10% the normal rate (C.S.N., unpublished observations). When the experiment is done at 38° C with *cdc*7, 149/150 DNA molecules were simple linear structures[4,5].

Hartwell[10] has pointed out the difficulties in distinguishing true DNA initiation mutants from mutants which have a leaky replication block, a replication block with a delayed onset of expression or a replication block early in the process of DNA replication. The observation that very few of the DNA molecules observed in DNA isolated from the initiation mutants *cdc*4 and *cdc*7 contain DNA replication intermediates argues that the cells are blocked before the onset of DNA replication. These data and the incorporation data from previous studies[8,9] indicate that *cdc*4 and *cdc*7 may be true DNA initiation mutants.

The findings with *cdc*8, the DNA propagation mutant, were quite different. Approximately half (114/240) of the DNA molecules isolated from *cdc*8 at the restrictive temperature contained one or more replication bubbles. Often these bubbles were very small, less than 2 μm in size (Fig. 1). Figure 2 is a histogram of bubble sizes. The average bubble (3 μm) observed in this experiment is less than that found in replicating molecules isolated from a synchronous early S-phase population of cells[4,5]. Figure 3 is a histogram of centre-to-centre distances between bubbles in molecules which contain more than one bubble.

The high proporation of DNA molecules with replication bubbles found in experiments with *cdc*8 indicate that this mutant can initiate DNA synthesis at the restrictive temperature. The spacings between adjacent bubbles (Fig. 3) are similar to those observed during normal DNA replication[4,5] suggesting that DNA synthesis may be initiating in *cdc*8 at the restrictive temperature at the same sites as during normal DNA replication. The small size of the replication bubbles probably reflects a much reduced rate of chain propagation at the restrictive temperature. The rate of nuclear DNA synthesis in *cdc*8 at the restrictive temperature is less than 1% the normal rate (C.S.N., unpublished observations). An alternative explanation for the small replication bubbles is that the *cdc*8 gene function is not required for replication of a small amount of DNA at the initiation site but is required for subsequent chain propagation. This explanation seems somewhat unlikely but cannot yet be ruled out. The small proportion of molecules with large replication bubbles may be molecules which contained replication intermediates at the time of the shift to the restrictive temperature (as discussed in the legend to Fig. 1). Therefore, the large number of DNA molecules in *cdc*8 at the restrictive temperature which contain small replication bubbles confirms the classification of *cdc*8 as a DNA propagation mutant. Hartwell[9] has recently classified another cell cycle mutant, *cdc*21, as a possible DNA propagation mutant on the basis of incorporation kinetics. Preliminary

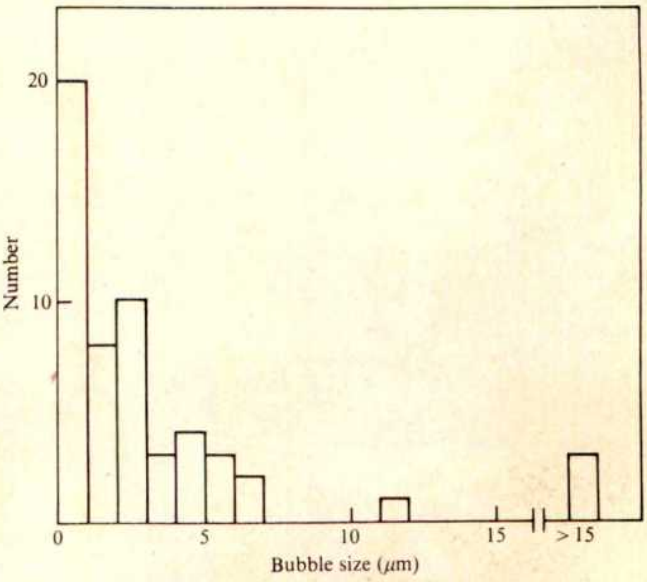

Fig. 2 Histogram of bubble sizes from DNA molecules isolated from *cdc*8 at the restrictive temperature. Photographs were printed at a final magnification of 10,000 to 25,000 and contour measurements were made with a Keuffel and Esser map measurer.

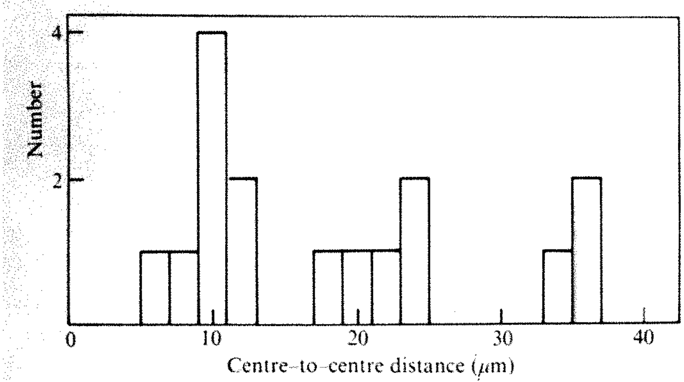

Fig. 3 Distribution of centre-to-centre distances between bubbles on molecules containing more than one bubble. Measurements were made on DNA molecules isolated from *cdc8* at the restrictive temperature.

experiments with *cdc21* at the restrictive temperature have shown that these DNA molecules also contain small replication bubbles (T.D.P., unpublished observations).

The appearance of small replication bubbles in the elongation mutant *cdc8* but not in the initiation mutants *cdc4* and *cdc7* cannot be explained by suggesting that *cdc8* is more leaky at the restrictive temperature than *cdc4* or *cdc7*. As already discussed, at 36° C, *cdc7* is considerably more leaky than *cdc* 8. These results, therefore, also imply a qualitative difference in the leakiness of *cdc7* and *cdc8* (ref. 10). In *cdc8* at the restrictive temperature, a high proportion of cells are apparently synthesising DNA at a slow rate of chain propagation (since many of the DNA molecules contain small bubbles). In *cdc7* at the restrictive temperature, the leakiness of synthesis may be the result of a small proportion of cells which escape the initiation block and replicate their DNA at a normal or nearly normal rate of chain propagation (since very few of the DNA molecules contain DNA replication intermediates).

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Radiological mapping of the ribosomal RNA transcription unit in *E. coli*

THE ribosomal RNA genes of *Escherichia coli* are arranged in tandem behind a common promotor in the order of 16S, 23S, 5S (for review see ref. 1). In this report we redetermine the positions of the 16S (0.55×10^6 dalton), 23S (1.10×10^6 dalton), and 5S rRNA (0.04×10^6 dalton) cistrons on their unit of transcription (scripton) by the ultraviolet light mapping technique[2–9] principally to demonstrate the applicability of this technique to the determination of the transcriptional linkage of genes in *E. coli*.

The ultraviolet light technique for mapping transcriptional linkage is based on the observation that certain lesions induced in DNA by ultraviolet light cause premature termination of transcription at the site of the lesion and that no reinitiation occurs beyond the lesion up to the next promotor[5,6,10,11]. Therefore, the expression of promotor distal genes is more sensitive to ultraviolet irradiation than that of promotor proximal ones.

Rates of synthesis of 23S, 16S and 5S rRNA after irradiation of *E. coli* with various doses of ultraviolet light were determined by ^{3}H-uridine pulses and quantitation of the RNA species after polyacrylamide gel electrophoresis. Errors originating with the isolation procedure were minimised by providing internal standards through prelabelling of the mature rRNAs with ^{14}C-uridine. In Fig. 1 (left and right panels) frames (*a*) display rRNAs synthesised in the unirradiated sample, frames (*b*) to (*f*) (left panel) and (*b*) to (*d*) (right panel) the synthesis in ultraviolet-light-irradiated cultures in the order of increasing dose. As Fig. 1 shows, synthesis of 23S rRNA was reduced more by ultraviolet light irradiation than was the synthesis of 16S rRNA. Synthesis of 5S rRNA was very sensitive to ultraviolet light. A greater reduction in 23S rRNA synthesis compared with 16S rRNA synthesis after ultraviolet irradiation has been observed previously[12–14].

Evaluation of a series of such experiments leads to the dose-effect curves in Fig. 2. The straight line parts of the curves, which extrapolate to 160 and 170% at the ordinate, yield inactivation coefficients of -3.7×10^{-2} s^{-1}, -0.12 s^{-1}, and -0.12 s^{-1} for synthesis of 16S, 23S and 5S rRNA, respectively. The ratio of 16S *versus* 23S rRNA synthesis as a function of dose is given in the insert of Fig. 2. We have utilised this RNA ratio curve in determining the extent of photoreactivation of RNA chain terminating lesions.

Inactivation curves for 16S, 23S and 5S rRNA synthesis all have shoulders in a semi-log plot, but with ultraviolet doses greater than 10 s they all enter into a straight line portion (Fig. 2). From this it can be concluded that all six rRNA transcription units[1] in a single *E. coli* chromosome are structurally identical.

We suggest that the shoulders of the survival curves are caused by the following effect. Not all ribosomal RNA scriptons are transcribed at the maximal rate in the unirradiated cell. As the number of unhit scriptons decreases within a cell with increasing ultraviolet dose, dormant rRNA scriptons become activated, or the remaining rRNA scriptons are utilised better. When cells are irradiated for more than 10 s the rates of rRNA synthesis are exclusively determined by the number of unhit cistrons for the mature rRNA species.

16S, 23S and 5S rRNA can be positioned fairly accurately on p30, the precursor RNA containing the mature rRNA species, if the inactivation coefficients and molecular weights of

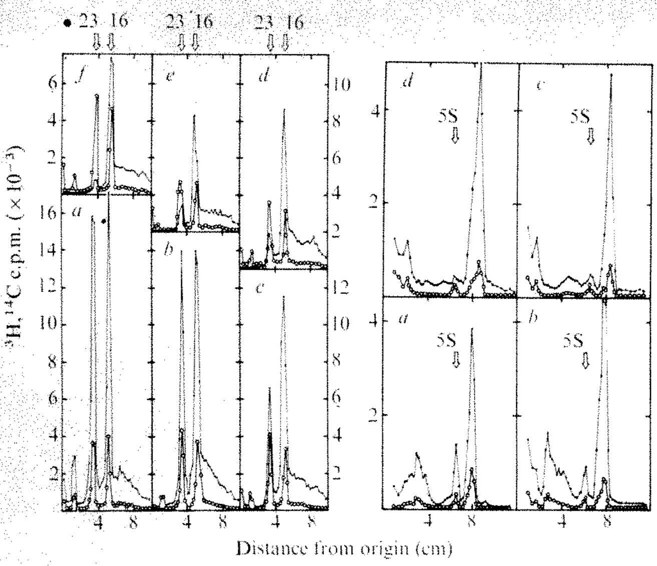

Fig. 1 Left panel: tritiated uridine labelling of 16S and 23S rRNA as a function of ultraviolet irradiation dose. A 20 ml culture of the ultraviolet-light-sensitive strain *E. coli* B_{s-1} (ref. 24) was grown in M-9 medium (M-9 plus 5% (w/v) casamino acids) at 37° C. At a concentration of 0.5×10^8 cells per ml, ^{14}C-uridine (54 mCi mmol^{-1}, Amersham-Searle) was added (0.01 µCi ml^{-1}) and cells were grown for approximately two more generations, about 90 min. This prelabelled culture was chilled on ice, centrifuged (5,100*g*, 15 min), resuspended in 30 ml fresh M-9 medium (approximately 10^8 cells ml^{-1}) and incubated for another 45 min at 37° C. The culture was then chilled on ice, split into six equal 5 ml portions and all but one irradiated at 0° C with a GE G4T4/1 low pressure mercury lamp for various times (incident dose rate at the position of the sample was 14 erg mm^{-2} s^{-1}, the effective dose rate was 10.5 erg min^{-1} s^{-1}). The unirradiated and five irradiated samples were transferred to culture tubes and incubated with vigorous shaking for 5 min. Tritiated uridine (42 Ci mmol^{-1}, Amersham-Searle) was added to a concentration of 1 µCi ml^{-1} to each culture. At 12.5 min after ^{3}H-uridine addition, the cultures were poured into ice cold centrifuge tubes, the cells were pelleted, resuspended in 2 ml 3mM EDTA (*p*H 7.2) and lysed by addition of 0.2 ml 10% Na dodecyl sulphate (SDS, Biorad) in a 1 min, 80° C incubation. RNA was extracted from the lysates with H_2O-saturated phenol, precipitated and resuspended in 0.30 ml 3 mM EDTA. To these RNA preparations sucrose was added to 15% and SDS to 1%. Samples of 0.10 ml of each RNA preparation were layered onto 15 cm 2.6% acrylamide gels and electrophoresed at 70 V for 5.5h; the gels were sliced into 2 mm fractions, digested in peroxide and counted in a liquid scintillation counter. The counts in the ^{3}H-pulse-labelled RNA and ^{14}C-prelabelled RNA were corrected for interchannel spillover. Right panel: tritiated uridine labelling of 5S and 4S RNA as a function of ultraviolet irradiation dose. Samples of 0.10 ml of the same RNA preparations used in the left panel were layered on to 8.0% polyacrylamide gels and electrophoresed at 75 V for 6 h in order to resolve the 5S and 4S RNAs. The major peak to the right of 5S rRNA is the 4S tRNA fraction. In both panels the ultraviolet irradiation times were (*a*) 0 s; (*b*) 8 s; (*c*) 15 s; (*d*) 23 s; (*e*) 31 s; (*f*) 38 s. No tritium counts in the 5S region were detectable at irradiation times in excess of 23 s (data not shown). ●, ^{3}H c.p.m.; ○, ^{14}C c.p.m.

the rRNAs are known. Since synthesis of the 23S is 3.25 times more sensitive than that of 16S rRNA, its 3′ terminus must be located at least $3.25 \times 0.55 \times 10^6$ daltons $= 1.80 \times 10^6$ daltons away from the p30 promotor. Since the molecular weight of the 5S rRNA is 0.04×10^6 and since 5S synthesis has essentially the same inactivation coefficient as 23S (Fig. 3), 5S must follow the 23S very closely on the transcriptional map. Thus, by converting ultraviolet-light-inactivation coefficients into molecular weights, using the molecular weight of 16S rRNA as a standard, and assuming the 16S RNA cistron to be adjacent to the promotor locus, we project a molecular weight of about 1.85×10^6 for p30. This size is close to previous determinations of the p30 molecular weight between 1.75 and 10^6 (ref. 15) and 1.85×10^6 (ref. 16). If this size for p30 is correct, our data would locate the 16S, 23S and 5S rRNA as shown in Fig. 3*a*, with the 3′ terminus of p30 at about 1.85×10^6 daltons. If the molecular weight of p30 were about 2.1×10^6 (ref. 17) the 16S rRNA could be preceded by approximately 0.09×10^6 daltons of non-conserved spacer RNA and the second spacer situated between 16S and 23S would be about 0.30×10^6 daltons (Fig. 3*b*). Alternatively, the additional 0.25×10^6 daltons of non-conserved RNA could be located beyond the 3′ end of the map as drawn (dashed line) in Fig. 3*a*. Figure 3*a* and *b* gives the extreme positions of 16S, 23S and 5S rRNA on p30. Any intermediate position that places the 3′ end of the 23S at 3.25 times the distance of the 16S 3′ end from the promotor would be consistent with our data.

From the inactivation coefficient for the synthesis of 16S rRNA, the dose required to produce 1 transcription terminating

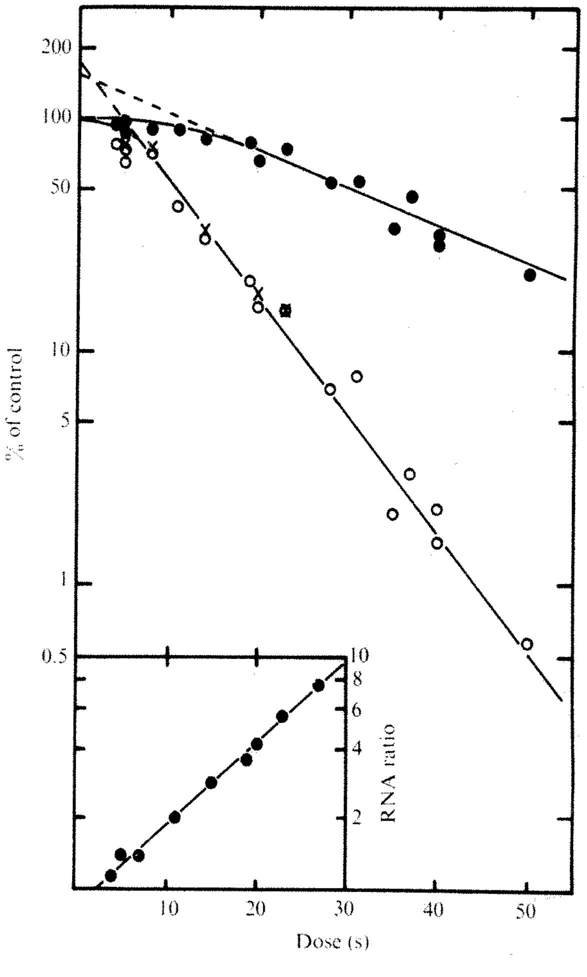

Fig. 2 Reduction of synthesis of rRNA species as a function of ultraviolet light dose. Data are taken from several experiments of the type shown in Fig. 1. The relative amount of 23S, 16S (converted from 17S values) and 5S rRNA can be calculated accurately, using the ^{14}C-prelabelled rRNA as an internal standard, from the expression:

$$\% \text{ of control synthesis} = \left[\frac{^{3}\text{H c.p.m. RNA}_x}{^{14}\text{C c.p.m. RNA}_x}\right]_d \cdot \left[\frac{^{14}\text{C c.p.m. RNA}_x}{^{3}\text{H c.p.m. RNA}_x}\right]_{\text{control}}$$

where *x* refers to the species of rRNA under consideration, the RNA in the brackets subscribed with a *d* is from a culture irradiated with a dose *d*, and the RNA in the brackets subscripted with 'control' is from the unirradiated culture. Background radioactivity due to non-rRNA was subtracted from the total radioactivity in the peak fractions for 23S, 17S and 5S rRNA. The exponential slopes, determined from least squares analyses of all points greater than 10 s, were $K_{16} = -3.7 \times 10^{-2}$ s^{-1}, $K_{23} = K_5 = -0.120$ s^{-1}. The ordinate intercepts of the exponential portions of the RNA synthesis dose-effect curves for 16S and 23S rRNA were 160% and 170% respectively. ●, 16S rRNA; ○, 23S rRNA; ×, 5S rRNA. The inset shows the increase in 16S rRNA synthesis relative to 23S rRNA synthesis (the RNA ratio) with increasing ultraviolet light dose.

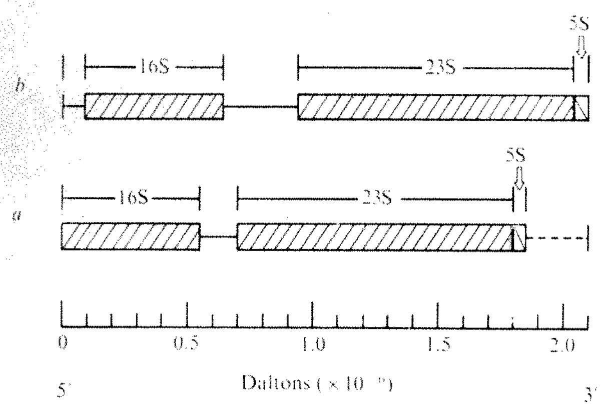

Fig. 3 Map of the *E. coli* ribosomal RNA transcription unit determined by radiological mapping. Two possibilities for the arrangement of the rRNAs (hatched areas) and non-conserved spacer RNAs (solid or dashed lines) are shown; model *a* assumes the molecular weight of p30 is 1.85×10^6 (refs 15 and 16) and model *b* assumes the molecular weight is 2.1×10^6 (ref. 17).

lesion per 1,000 DNA base pairs in the p30 scripton was determined to be between 550 and 640 erg mm^{-2}.

From the extent to which ultraviolet-light-induced transcription terminating lesions in DNA can be eliminated by photoreactivation, one can estimate what fraction of these lesions comprises pyrimidine dimers. After maximal photoreactivation, 70% of the transcription terminating lesions were repaired as determined from the shift in the ratios of 16S to 23S rRNA (ref. 7 and inset, Fig. 2). This suggests that at least 70% of the transcription terminating lesions comprises pyrimidine dimers[18]. Photoreactivation of ultraviolet-light-induced lesions which impair RNA synthesis in general has been reported earlier[19].

Actinomycin D has been used in studies of transcriptional linkage[20,21] with the same rationale as we use in the ultraviolet light mapping technique. The ribosomal genes of *E. coli*, however, were incorrectly assigned to separate transcription units through the actinomycin D technique[20], and the transcriptional linkage of the β-galactosidase and transacetylase genes of the *lac* operon of *E. coli* (ref. 22) was determined correctly only through an error in evaluating the experimental data. The transcriptional arrangement of the rRNAs in mouse L cells was incorrectly inferred from actinomycin D inhibition of RNA synthesis (see ref. 7). Thus far, actinomycin D has failed as an agent for transcription mapping in both prokaryotes and eukaryotes[7], whereas ultraviolet light mapping has been used successfully in both systems (for review see ref. 23).

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Distribution of DNA in dividing spinach chloroplasts

SPINACH chloroplasts, like other higher plant chloroplasts, contain DNA[1] and divide[2]. It has been estimated[3-5] that chloroplasts contain many copies of DNA, but in higher plants, which contain large numbers of chloroplasts per cell[2,6] it is not known when the DNA of chloroplasts replicates and how it is distributed when they divide. In the unicellular alga, *Ochromonas*, which contains a single chloroplast, ^{3}H-thymidine is readily incorporated into chloroplast DNA and is distributed equally among the progeny chloroplasts[3]. On the other hand, in *Acetabularia*, which contains large numbers of chloroplasts per cell, DNA could not be detected in 65–80% of the plastids, but could easily be detected in the remainder[7].

Disks of spinach leaves grown in sterile culture were used to study the distribution of DNA in dividing chloroplasts in both pulse and pulse/chase experiments. In this system chloroplast number per cell increases 5–10-fold over a 7 d culture period in the light[2]. There is virtually no cell division during this time[2].

Using ^{3}H-thymidine and autoradiography on EDTA-separated cells dried on to glass slides (Fig. 1) we found that in spinach leaf discs both chloroplast and nuclear DNA synthesis continues during cell expansion. The specificity of incorporation into DNA was established using DNase, RNase and acid extractions[8].

The distribution of label amongst daughter chloroplasts was followed in freshly excised spinach disks (2 mm dia-

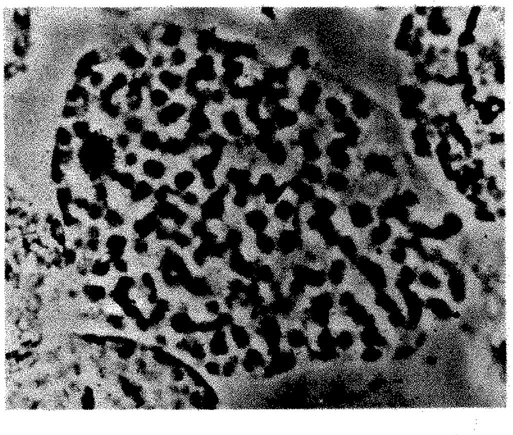

Fig. 1 Autoradiograph of leaf cell showing ^{3}H-thymidine incorporation. Tissue was incubated in ^{3}H-thymidine for 24 h and chased 4 d as indicated in Table 1 (×400).

Table 1 ^{3}H-Thymidine incorporation into spinach chloroplasts

	End of pulse	End of chase
Grains per chloroplast	23.9	4.5
Labelled chloroplasts	100%	99%
Chloroplasts per cell	23	148

Spinach disks 2 mm in diameter were excised from the base of leaves 2 cm long and grown on sterile agar[4]. 1 ml of 50 μCi 6-^{3}H-thymidine (27 Ci mM^{-1}, The Radiochemical Centre, Amersham, UK) was added under sterile conditions to the 20 ml of medium in 9 cm Petri dishes. The tissue was incubated for 24 h (14 h d^{-1}, 20,000 l×) in a growth cabinet. After incubation, nine disks were fixed in 3% glutaraldehyde after a rinse in 2.07 mM cold thymidine. The other nine disks were transferred to fresh medium (see text), containing approximately a 1,000 times excess of cold thymidine. After 4 d in the fresh medium, the nine remaining disks were fixed in 3% glutaraldehyde. The disks from both the pulse and chase were treated with 0.05 M EDTA (pH 9) overnight at 60° C to separate the cells. The cells were spread on to slides, air dried and dipped in Ilford L4 nuclear emulsion. The slides were exposed 13 d at 5° C before developing and mounting as permanent slides. The data for the pulse was obtained from 10 cells with all plastids scored as labelled or unlabelled, and 10 plastids from each cell scored for grain number. The chase data was obtained in the same way except that 20 plastids per cell were scored for grain number. Independent counts were made by two observers and only chloroplasts clearly separated from each other were scored. Background corrections were made; these varied from zero to two grains per chloroplast.

meter) incubated in ^{3}H-thymidine for 24 h. At the end of the incubation period, one half of the disks were collected for autoradiography, and the other half rinsed with sterile water and transferred to fresh medium containing an excess of cold thymidine for a further 4 d growth period.

At the end of the ^{3}H-thymidine pulse there were 23 plastids per cell and this increased to 148 at the end of the chase (Table 1). This represents approximately 2.7 generations. Almost all plastids were labelled both at the end of the pulse and at the end of the chase (Table 1). These results indicate that the label is distributed to virtually all daughter chloroplasts. The mean number of grains decreased from 23.9 at the end of the pulse to 4.5 at the end of the chase (Table 1), which is close to the 3.7 expected if the grain number was halved at each generation. This is consistent with the chloroplast DNA being conserved during the chase period. The nuclear DNA also appears to be largely conserved as there were 44% labelled nuclei at the end of the pulse and 36% at the end of the chase. We have also found a fourfold increase in the total DNA of discs, over a 6 d growth period, even though there is no significant cell division during this time.

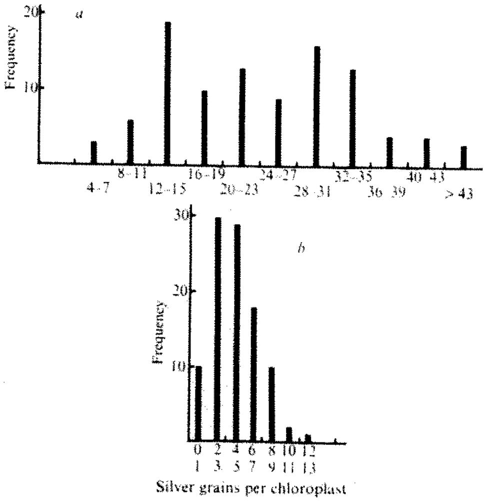

Fig. 2 Frequency distribution histograms of silver grains over a 100 chloroplasts after, *a*, a 24-h pulse in ^{3}H-thymidine, and, *b*, a 4-d chase as indicated in Table 1. The observed distribution of the pulse differs from that of the chase. The distribution of the chase does not differ significantly from a Poisson distribution having the same mean (chi-square test: $P > 0.05$). An autoradiograph of a cell after the chase is shown in Fig. 1.

The distribution of silver grains at the end of the pulse probably reflects the different times at which plastids completed their last division (Fig. 2). No highly labelled chloroplasts were found at the end of the chase and the labelling pattern corresponds closely to a Poisson distribution. This is good evidence that the label distributes almost equally amongst daughter chloroplasts as is the case in *Ochromonas*[3].

Our results differ from those of Woodcock and Bogorad[7] with *Acetabularia* where there are large variations in the DNA content of chloroplasts. Herrmann[9,10] has found, using chloroplast preparations, isolated from whole or parts of *Beta vulgaris* leaves, that large chloroplasts contain more DNA than small chloroplasts. He did not look at variations in the DNA content of chloroplasts in single cells. Some of the variation in DNA amongst chloroplasts of different sizes can be related to developmental and nuclear genotype changes. We consider these differences are unrelated to the segregation of DNA that occurs in a rapidly dividing chloroplast population which is concomitantly synthesising DNA.

The data we have obtained establish that there is active chloroplast DNA synthesis in expanding leaf cells of spinach and that chloroplast DNA segregates to all daughter chloroplasts during chloroplast replication. The data further suggest that all chloroplasts of the cell divide rather than a small subpopulation as has been proposed from chloroplast size and observations[6].

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Friend virus release and induction of haemoglobin synthesis in erythroleukaemic cells respond differently to interferon

EXPERIMENTS with intact cells have provided the firmest evidence that the synthesis of viral proteins is the main function inhibited in cells treated with interferon. Changes

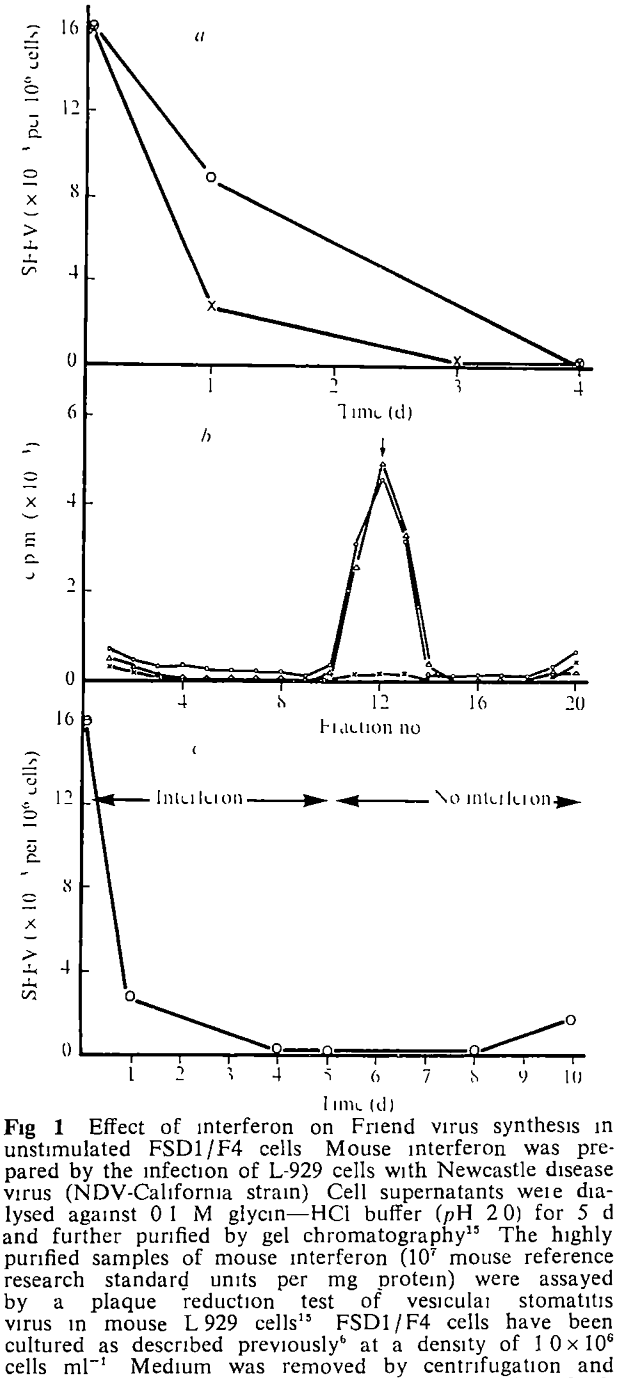

Fig 1 Effect of interferon on Friend virus synthesis in unstimulated FSD1/F4 cells Mouse interferon was prepared by the infection of L-929 cells with Newcastle disease virus (NDV-California strain) Cell supernatants were dialysed against 0 1 M glycin—HCl buffer (*p*H 2 0) for 5 d and further purified by gel chromatography[15] The highly purified samples of mouse interferon (10^7 mouse reference research standard units per mg protein) were assayed by a plaque reduction test of vesicular stomatitis virus in mouse L 929 cells[15] FSD1/F4 cells have been cultured as described previously[6] at a density of $1\ 0 \times 10^6$ cells ml^{-1} Medium was removed by centrifugation and cells transferred to the same volume of either fresh medium or fresh medium containing 300 or 900 U ml^{-1} mouse interferon, respectively Cells were transferred in daily intervals to fresh medium with or without interferon After removal of the cells, aliquots of cell supernatants were assayed for SFFV in DBA-2 mice[16] Total synthesis of C type particles was analysed by measurement of ^3H uridine incorporation into cell supernatant particles banding at 1 16–1 18 g cm^{-3} Cells were exposed to either 600 U ml^{-1} mouse interferon or 500 U ml^{-1} chicken interferon from 0–48 h and 25 $\mu Ci\ ml^{-1}$ 5,6 ^3H-uridine (Amersham, specific activity, 50 Ci $mmol^{-1}$) from 24–48 h Cell supernatants were cleared from debris at 8,000*g*, 10 min and centrifuged at 60,000*g* 1 h 4° C Pellets were resuspended in a buffer containing 0 1 M NaCl, 0 01 M Tris-Cl, 0 001 M EDTA, *p*H=7 4, layered on top of a 15–60% (w/v) sucrose gradient in the same buffer and centrifuged for 75 min in a Spinco SW 50-1 rotor at 42,000 r p m Acid precipitable radioactivity was determined in each fraction *a*, Reduction of Friend virus (FV) release by interferon Spleen focus formation of supernatant particles ○, 300 U ml^{-1} interferon, ×, 900 U ml^{-1} interferon *b*, Reduction of FV release by interferon, uridine incorporation into viral particles, density 1 16–1 18 g cm^{-3} (↓), sucrose gradient ○, Untreated cells, ×, mouse interferon 600 U ml^{-1}, △, chicken interferon 500 U ml^{-1} *c*, Aftereffect of interferon treatment 900 U ml^{-1} treatment as in *a* Interferon-free medium from day 5 on

in an interferon-treated cell which lead to the development of resistance may be reflected also in a cell-free system for protein synthesis Thus, polyribosomes of interferon-treated L cells were found unable to translate either mengovirus mRNA or haemoglobin mRNA—in contrast to the endogenous mRNA synthesis which was not impaired[1] A similar inhibition of the translation of exogenous viral or cellular mRNAs was reported for cell-free extracts from interferon-treated Ehrlich ascites cells[2] These observations raise the question of whether the expression of some cellular functions is as equally affected as that of viruses The inhibition of haemopoietic colony growth and of phytohaemagglutinin stimulation of lymphocytes by interferon seems to indicate that interferon does have such a dual effect[3,4] We show here, however, that interferon inhibits virus release or replication but that it does not interfere with DMSO-induced differentiation

Friend virus-transformed erythroleukaemic spleen cells can be maintained in culture They can be induced to differentiate[5] and to synthesis haemoglobin[6] Some of the erythroleukaemic cell lines (clone FSD1/F4) liberate biologically active Friend virus (FV), that is, spleen focus forming virus (SFFV) (ref 7) and lymphatic leukaemia

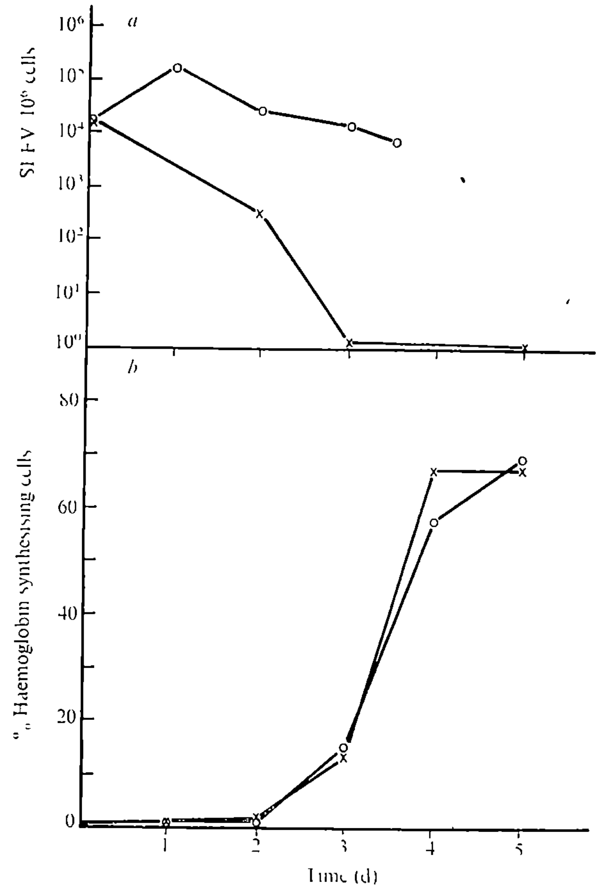

Fig 2 Effect of exogenous interferon on the production of FV (SFFV) and on haemoglobin synthesis during dimethylsulphoxide (DMSO)-induced differentiation of FSD1/F4 cells Stimulation of FSD1/F4 cell cultures by DMSO treatment has been described[6] Interferon was added to the culture medium at a concentration of 900 U ml^{-1} During the course of stimulation, cells were transferred daily to fresh medium containing 1 5% DMSO with or without interferon The production of SFFV was tested at various intervals during stimulation as described previously[16] Erythroid differentiation of cells was estimated by staining cells with benzidine *a*, Effect of interferon on virus synthesis in DMSO stimulated cells ○, DMSO 1 5%, no interferon, ×, DMSO 1 5%, 900 U ml^{-1} mouse interferon *b*, Lack of effect of interferon on haemoglobin synthesis The % of benzidine-positive cells is plotted ○, DMSO 1 5%, no interferon, ×, DMSO 1 5%, 900 U ml^{-1} mouse interferon.

virus (LLV) LLV acts as a helper in spleen focus formation This cell line can therefore be used to study the effect of exogenous homologous (mouse) interferon on the release of C-type viruses and on the induction of erythroid differentiation (haemoglobin synthesis)

Interferon inhibits tumour formation by RNA tumour viruses[8,9] In some instances, however, interferon can be used to enhance neoplastic development[10] Interferon also interferes with the transformation of fibroblasts by sarcoma viruses[11] Mice with Friend erythroleukaemia have been reported to display a reduced capacity to respond to inducers of interferon[12 13] No interferon could be detected in the supernatants of FSD1/F4 cells or two other lines of SFFV-transformed cells when proliferation was either in the absence or presence of dimethylsulphoxide (DMSO), indicating that FV does not trigger the interferon response in these cells Although interferon production can be induced in these cells by an exogenous viral inducer (Newcastle disease virus) no response to poly(I) poly(C) (ref 12) was observed (P S and W O, unpublished) Similar observations on differential inducibility of interferon in transformed mouse cells were made by others (F Ruddle, unpublished)

The effect of interferon on FV release in unstimulated cells was demonstrated by the addition of 300–900 mouse reference research standard units (U) interferon ml^{-1} to cell clone FSD1/F4 We find a more than 100-fold reduction both of spleen focus formation with cellular supernatants and of uridine label in C-type particles (Fig 1*a*, *b*) Heterologous chicken interferon does not reduce the virus yield (Fig 1*b*) The recovery of virus production after interferon treatment for 5 d and then the omission of interferon is slow and possibly incomplete Five days after the omission of interferon only 10% of the normal virus level is obtained (Fig 1*c*) The growth rate of the interferon treated cells was only slightly reduced

When FSD1/F4 cells are exposed to growth medium which contains 1–1 5% DMSO, not only the onset of erythroid differentiation but also a 5–10-fold increase in released virus particles during the first and second day of differentiation can be observed The titre of released virus increases 10–100-fold as assayed either by the XC-test (LLV) or by the spleen focus forming test (LLV+SFFV)[7] Interferon, however, depresses the virus synthesis below background level (Fig 2*a*) Differentiation as measured by globin synthesis is only slightly inhibited by 300–900 U ml^{-1} interferon If interferon is added 3 d before the onset of DMSO induced differentiation, perfectly normal differentiation is observed The course of differentiation, that is the number of benzidine positive cells after adding DMSO or DMSO and interferon, remains unchanged (Fig 2*b*) The amount of globin synthesised after 4 d stimulation with DMSO is not reduced by adding 300–900 U ml^{-1} interferon (Fig 3)

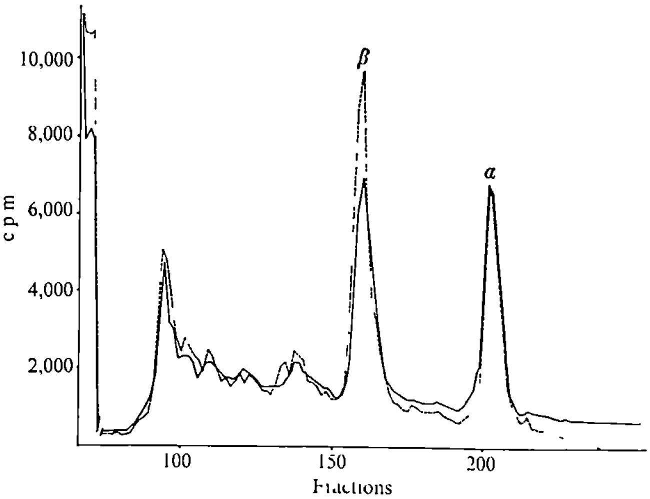

Fig 3 Stimulation of globin synthesis with 1 5% DMSO, Effect of interferon Cells were pretreated with interferon 900 U ml^{-1} for 3 d DMSO (1 5%) was then added and incubation with DMSO+interferon continued for another 4 d Medium was replaced daily Cells were labelled with ^{3}H- or ^{14}C-leucine in leucine-free medium for 16 h Extraction of NP40 soluble cytoplasmic proteins and separation of globin chains on carboxy methylcellulose columns was done as described previously[6] ----, DMSO 1 5% no interferon, ^{14}C-leucine, ——, DMSO 1 5% 900 U ml^{-1} mouse interferon, ^{3}H-leucine

As the degree of purity of interferon often reflects on the meaningfulness of results we confirmed our data obtained with partially purified interferon (1 5×10^{5} U mg^{-1} protein), by using a highly purified interferon preparation (10^{7} U mg^{-1} protein) We found that within our limits the degree of purity had no effect on the interferon action

The data above make possible, for the first time, the differentiation between the effect of exogenous interferon on C-type virus release in transformed cells and the effect on induced differentiated cellular functions in the same cell While virus synthesis is strongly inhibited, differentiation remains unaffected Interferon neither changes the time course nor the amount of haemoglobin synthesis It does not change the number of cells involved in haemoglobin synthesis either We conclude that the release of Friend virus is not required for *in vitro* erythroid differentiation of these cells

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Erythropoietin responsiveness of differentiating Friend leukaemia cells

INCLUSION of dimethylsulphoxide (DMSO) in the media of suspension cultures of Friend leukaemia cells (FLC) results in the erythroid differentiation of more than 50% of the cells[1] This differentiation is demonstrated by morphological and biochemical alterations which are characteristic of normal erythroid maturation[2-5] We report here that the differentiation of these leukaemic cells is accompanied by the development of responsiveness to erythropoietin (Ep), the physiological regulator of erythropoiesis

Friend leukaemia cells (clone 745A) were grown in suspension culture in media consisting of basal Eagle's medium with Earl's balanced salts (GIBCO), made 15% with foetal calf serum (GIBCO) at 37° C in a humidified atmosphere of 5% CO_2 and 95% room air The cultures were passaged at 3 or 4 d intervals at a seeding concentration of 1×10^5 cells ml^{-1} DMSO (Fisher Sci) was added (2% v/v) where appropriate at the time of seeding the cultures Erythropoietin (human urinary erythropoietin 75 4 IU mg^{-1}) was added to the suspension cultures at the times indicated

To assess the rate of haem synthesis, 0 5 μCi of mouse transferrin-bound ^{59}Fe was added to culture plates containing 3 ml of the suspension culture and after 4 h further incubation the cells were collected, washed twice with isotonic saline and the haem extracted with cyclohexanone according to the method of Krantz[6], and counted This procedure provides an accurate assessment of the rate of haem synthesis by Friend leukemia cells[7] We have already shown that during DMSO-induced differentiation of Friend erythroleukaemia cells, increased iron incorporation into haem was coordinated with increases in the rate of synthesis of globin, haemoglobin, and globin mRNA[8] To assess the rates of DNA, RNA and protein synthesis, 5 μCi of ^{3}H-thymidine, ^{3}H-uridine or ^{3}H-leucine (New England Nuclear Co) were added to appropriate culture plates containing 4 ml of suspension cell and after 40 min of incubation the cells were collected, washed twice, and the rate of precursor incorporation determined according to the method of Schneider as discussed by Shatkin[9] All radioactive assays were determined using three plates for each experimental point and each experiment was carried out at least three times

Ep was added to suspension cultures of erythroleukaemia cells which had previously been grown in the presence of DMSO for 3 d (Table 1) This significantly stimulated the rate of haem synthesis of the cells Ep had no significant effect on the rate of increase in cell numbers in the culture (which increased from 0 95±0 03×10^6 cells ml^{-1} on day 3 to 1 24±0 04×10^6 cells ml^{-1} on day 4) nor did it result in an increase in the proportion of benzidine-positive cells in the culture Ep had no effect on the rate of haem synthesis by cells which had not been cultured in the presence of DMSO (Table 2)

The stimulatory effect of Ep on DMSO-treated cells was not limited to haem synthesis The effects of Ep on the rate of precursor incorporation into DNA, RNA and protein are given in Table 2 The increases in leucine and uridine incorporation were quantitatively similar to that observed for ^{59}Fe, the rate of DNA synthesis also increased These effects of Ep on the rates of macromolecular synthesis by these differentiating leukaemic cells were similar to those reported for normal rat bone marrow cells[10] and foetal liver erythroid cells[11] Although Ep had no effect on the rates of haem, RNA or protein synthesis by cells which had not been cultured in the presence of DMSO, the addition of Ep to suspension cultures of these cells resulted in a decrease in the rate of thymidine incorporation into DNA

During cell growth and replication, Ep responsiveness could be reliably detected when Ep was added on the third day of culture and haem synthesis assayed 1 d later The addition of Ep earlier during culture with assay of haem synthesis 24 h after addition produced no significant effect The addition of Ep to cultures which had been growing in the presence of DMSO for more than 3 d also resulted in an increase in the rate of haem synthesis By contrast, cultures to which Ep was added at the time of seeding in the presence of DMSO synthesised less haem on the fourth day of culture than cultures also seeded in the presence of DMSO but to which Ep was not added In six such experiments the rate of haem synthesis (mean c p m per plate ± s e for 17 degrees of freedom) was 16±4 8% less than cultures seeded without Ep

Table 1 Effect of Ep on erythroleukaemic cells grown in DMSO medium

Parameter	Mean % stimulation ± s e m	No experiments
(1) Haem synthesis		
a CPM-^{59}Fe per plate	+29 ± 4 6	29
b CPM-^{59}Fe per 10^6 cells	+29 ± 4 1	29
(2) Cell number	+3 ± 2 4	29
(3) % Benzidine-positive cells	+6 ± 4 7	20

Tissue culture plates were set up containing 4 ml of suspension culture which was made 2% (v/v) with DMSO at the time of seeding After 3 d of culture Ep (0 06 IU ml^{-1}) was added to appropriate plates and 24 h later, after removal of 1 ml from each plate for cell counts and morphological studies, ^{59}Fe was added and 4 h later the haem extracted For morphological studies, slides were made with a cytocentrifuge, were benzidine stained[13], and counterstained with Wright-Giemsa The proportion of benzidine-positive cells was determined by a single observer in a single-blinded position The following equation was used to determine % stimulation of each parameter

$$\frac{(\text{Parameter Ep culture}) - (\text{Parameter no Ep culture}) \times 100}{(\text{Parameter no Ep culture})}$$

The % stimulation was determined for each individual experiment and the data so derived pooled and used to calculate the grant mean and s e m for each parameter

Ep was an effective stimulant of haem synthesis within a broad range of concentrations A comparison of the stimulatory effects of 0 04 IU ml^{-1} and 0 2 IU ml^{-1} on the rate of haem synthesis revealed no significant difference (34 5±6 4% stimulation for 0 04 IU ml^{-1} compared with 44±6 6% stimulation for 0 2 IU ml^{-1})

We have tested three different human urinary Ep and three different Step III sheep plasma Ep and have found no significant differences in their effects

In a preliminary communication we reported that Ep stimulated the rate of haem synthesis by Friend leukaemia cells which had previously been cultured in the presence of DMSO[12] In those studies, however, the condition of culture were such that the cells were not replicating and the rate of haem synthesis in all the cultures was declining The studies reported here demonstrate that under conditions of growth the erythroid differentiation of these leukaemic cells is accompanied by the development of responsiveness to Ep It is the process of differentiation *per se* and not the penetrant carrier properties of DMSO, that is responsible for the responsiveness to Ep, since Ep stimulated the rate of haem synthesis when the differentiating cells had been transferred to fresh medium before adding Ep[12] It is of interest in this regard that under the conditions of culture reported here, the appearance of responsiveness to Ep correlated with a major increase in the proportion of recognisable differentiating cells in the culture (the percentage of benzidine-positive cells on days 1 and 2 of culture were 1% and 5%, whereas on the third day it was 50%) This suggests that under these conditions Ep affects cells which have differentiated to the point of having synthesised enough haem to be recognisable by benzidine staining The effects of Ep on normal erythroid precursor cells which are more differentiated than the committed erythroid stem cell have been reported by others[13-15] The apparent inhibitory effects of Ep on DNA synthesis by control cells and on the rate of haem synthesis by DMSO-treated cells when Ep was added at the time of seeding was unexpected and may have been due to other substances present in the preparation of human urinary Ep The resolution of this question awaits the availability of pure Ep

Table 2 Effects of Ep on synthesis of DNA, RNA and protein

Macromolecule	Ep	DMSO-containing cultures Mean c p m per 10^6 cells ± s e	Control cultures Mean c p m per 10^6 cells ± s e
(*a*) DNA	0	6,073 ± 1182	3,928 ± 158
	+	7,474 ± 161	1,501 ± 34
(*b*) RNA	0	1,737 ± 145	5,018 ± 254
	+	2,556 ± 174	5,381 ± 137
(*c*) Protein	0	4,408 ± 84	15,687 ± 310
	+	6,653 ± 557	15,553 ± 428
(*d*) Haem	0	1,169 ± 128	7
	+	1,796 ± 103	7

Suspension cultures were set up in bottles containing 40 ml each After 3 d of culture Ep was added to appropriate bottles (0 06 IU ml^{-1}) and after 24 h of additional culture, the suspension cultures were distributed into triplicate plates for determination of the rate of macromolecular synthesis The differences between the rates of macromolecular synthesis between Ep and non-Ep containing cultures were statistically significant (P < 0 05) for all of the DMSO-containing cultures and for the decrease in the rate of DNA synthesis of the control cultures

All determinations were done in triplicate

These observations suggest that erythroleukaemic cells, and perhaps leukaemic cells in general, possess the genetic information needed for the development of responsiveness to normal physiological regulators and that under appropriate conditions this information is expressed Responsiveness to Ep by a continuous suspension culture cell line promises of facilitate studies of the mechanism of action of this hormone

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Growth of rust fungi of wheat and flax on chemically-defined media

THE rust fungi cause serious economic losses of wheat and other crops In nature, these organisms complete their life cycles only on living tissues of their hosts and, until recently[1], they have been regarded as obligate parasites due to their failure to grow in axenic culture on non-living substrates We have now obtained highly reproducible vegetative growth and sporulation of the flax rust fungus, *Melampsora lini* (Ehrenb) Lév (race 3) and vegetative growth of two North American races (15B-4 and 56) of the wheat stem rust fungus, *Puccinia graminis* f sp *tritici* (Eriks and Henn) on chemically defined liquid media, sterilised by millipore filtration and seeded with uncontaminated uredospores Vegetative growth and some sporulation were also obtained with an Australian isolate of wheat rust, race 126-ANZ 6, 7

Williams *et al*[2] reported the first successful establishment of axenic colonies of the wheat stem rust fungus, race 126-ANZ 6,7, starting with uncontaminated uredospores seeded on to an agar medium containing glucose, mineral salts, Evans's peptone and yeast extract Subsequently much progress has been made in the axenic culture of a number of rust fungi belonging to the *Pucciniaceae*[4-7] and *Melampsoraceae*[8-10] Nevertheless the results obtained suffer from a number of defects Most experimenters have employed agar media containing complex organic substances like peptone yeast extract and bovine serum albumin Only a few attempts[11-13] have been made to grow rusts on chemically defined media containing amino acids and growth on such media has not been equivalent to that obtained in the presence of peptone, yeast extract or bovine serum albumin Moreover, all the results reported thus far indicate that sporulation in axenic culture is erratic, and there are only two reports in which uredospores produced in axenic culture have been shown to be capable of reinfecting the host[3,6] Finally, the use of agar based media, sterilised by autoclaving, is itself a disadvantage, since the exact composition of the sterilised medium is not known Our objectives were to develop chemically defined media (see Table 1 legend) in order to examine the factors controlling sporulation and to compare the amino acid requirements of the wheat and flax rust fungi

Uncontaminated uredospores of both rusts were raised on susceptible hosts as described elsewhere[14] All media were sterilised, after the addition of amino acids, glutamine and glutathione as indicated in Table 1, by millipore filtration (pore size 0 22 μm) No agar was employed The pH of all media was adjusted to 5 0 Uredospores (1,000–2,000 mm^{-2}) were floated on the liquid surface (10 ml) in 50 ml flasks For each treatment five replicate flasks were incubated without shaking at 17° C in the dark The growth and development of the rust colonies were observed over a period of two months

The chief results are summarised in Table 1 Luxuriant growth of *M lini* was obtained on medium A with 45 mM aspartic or glutamic acid, plus cysteine (4 6 mM) and reduced glutathione (3 3 mM), or with 45 mM aspartic or glutamic

Table 1 Comparative behaviour of flax and wheat stem rust fungi *in vitro*

Fungus	Medium	Organic nitrogen source (mM) Aspartic acid*	Glutamine	Cysteine	Glutathione	Growth	Sporulation
Melampsora lini Race 3	A	45	0	4.6	3.3	Luxuriant	Frequent
	A	45	0	4.6 (or 2.3)	0	Luxuriant	Frequent
	A	0	42	4.6	0	Luxuriant	Nil
Puccinia graminis tritici Race 126-ANZ 6, 7	B	45	0	4.6	3.3	Luxuriant	Infrequent
	C	45	0	4.6 (or 2.3)	0	Luxuriant	Infrequent
	C	0	42	4.6	0	Luxuriant	Nil
	C	0	6	4.6	0	Poor	Nil
Race 15B-4 and Race 56	B	45	0	4.6	3.3	Nil	Nil
	C	45	0	4.6 (or 2.3)	0	Nil	Nil
	C	0	42	4.6	0	Luxuriant	Nil
	C	22.5	21	4.6	0	Luxuriant	Nil
	C	0	42 (Asp 45)	4.6	0	Luxuriant	Nil

*Aspartic acid can be replaced by equimolar glutamic acid with identical results. Asp 45 denotes that after 3 weeks' growth 42 mM glutamine was replaced by 45 mM aspartic acid. Basic components of media (l^{-1}): medium A: Sucrose, 50 g; $Ca(NO_3)_2 \cdot 4H_2O$, 2.0 g; KNO_3, 0.25 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; KH_2PO_4, 0.25 g; K_2HPO_4, 0.75 g; NH_4NO_3, 20 mg; 0.8 ml micronutrient solution (containing 13% NaFe (Geigy), 10 g; $MnSO_4 \cdot 7H_2O$, 447 mg; KI, 10 mg; $NiCl_2 \cdot 6H_2O$, 18 mg; $Ti(SO_4)_2 \cdot 9H_2O$, 42 mg; $ZnSO_4$, 35 mg; $CuSO_4 \cdot 5H_2O$, 15 mg; $BeSO_4$, 20 mg; H_3PO_4 (85%), 10 mg; H_2SO_4 (concentrated), 0.2 ml; all in 200 ml glass distilled water). Medium B: glucose, 30 g; $NaNO_3$, 2.0 g; KCl, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KH_2PO_4, 1.0 g; $Ca(NO_3)_2 \cdot 4H_2O$, 2 to 6 g; $FeSO_4 \cdot 7H_2O$, 10 mg, plus 0.8 ml micronutrient solution as in Medium A. Medium C: Major components as in Medium B, plus sodium citrate, 1.5 g; no micronutrients.

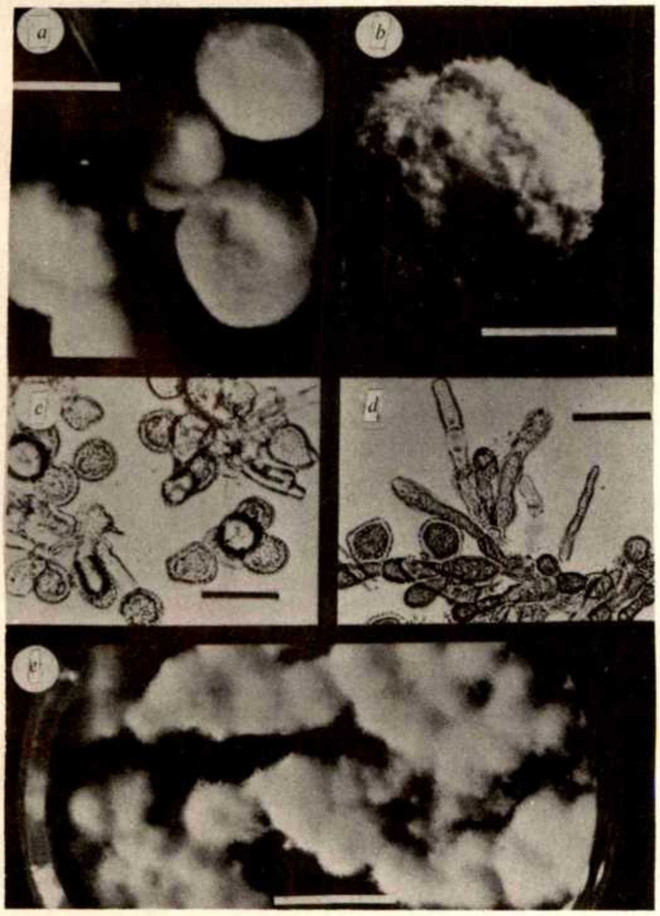

Fig. 1 Growth and development of *M. lini* (race 3) on liquid medium A (inorganic salts, sucrose, aspartic acid and cysteine). *a*, Fully developed vegetative colonies (1 month old) on liquid surface (scale bar, 0.5 cm); *b*, sporulating colony, 6 week old. The entire colony has developed into a uredosorus (scale bar, 0.5 cm); *c*, Uredospores (oblong cells) from colony like that in *b* (scale bar, 50 μm); *d*, teliospores from 2 month old colony (scale bar 50 μm); *e*, 1 month old vegetative colonies of *P. graminis* (race 15B-4) on liquid medium C (inorganic salts, glucose, glutamine and cysteine) (scale bar, 1 cm).

acid plus cysteine (2.3 or 4.6 mM). Sixty to seventy vegetative colonies per flask developed in 90–100% of the flasks within 4 weeks (primary axenic colonies). Two weeks later typical orange uredopustules were formed on the surfaces of most colonies (Fig. 1*a* and *b*) and dark brown teliosori appeared on some of them after 8 weeks growth. The uredospores and teliospores and their supporting hyphae were apparently closely similar to those found in nature (Fig. 1*c* and *d*). When uredospores produced *in vitro* were used to inoculate intact week-old flax cotyledons, infection and development of sporulating pustules occurred in a normal manner. When seeded on fresh medium, flax rust uredospores developed *in vitro* grew into secondary axenic colonies. These developed and sporulated in a manner indistinguishable from the primary axenic colonies. Uredospores from the secondary colonies also reinfected intact flax cotyledons. Decreases in the levels of aspartic or glutamic acid (22.5 mM) and cysteine (2.3 mM) resulted in poor growth and a low frequency of initiation of colonies, which failed to sporulate; glutathione was not essential for growth or sporulation. Replacement of aspartic acid with a high level of glutamine (42 mM) gave a high rate of colony initiation and excellent vegetative growth. These colonies were white and fluffy and had a yellowish stroma on the under surface, but did not sporulate and finally died.

An Australian isolate of *P. graminis tritici*, race 126-ANZ 6,7, developed large colonies on medium B plus high levels of aspartic acid (45 mM), cysteine (4.6 mM) and glutathione (3.3 mM); small areas of uredospores and teliospores developed on a few colonies in about 50% of the inoculated flasks. When uredospores formed by these colonies were placed on exposed mesophyll tissue they produced typical infections of seedling leaves of wheat[14]. Identical results were obtained on medium C plus 45 mM aspartic acid and 4.6 mM cysteine, so that glutathione and micronutrients were evidently not essential for growth. Of the three races of wheat stem rust, 126-ANZ 6,7 therefore most closely resembles race 3 of *M. lini* in its requirement for amino acids. Two North American isolates, races 15B-4 and 56, behaved in an entirely different manner and completely failed to grow on these media (Table 1).

Races 126-ANZ 6,7, 15B-4 and 56 all formed large vegetative colonies on medium C plus high levels of glutamine (42 mM) and cysteine (4.6 mM), but no sporulation occurred. Races 15B-4 and 56 also formed luxuriant vegetative colonies but did not sporulate on medium C plus aspartic acid, glutamine and cysteine (Fig. 1*e* and

Table 1). When glutamine was replaced with aspartic acid after 3 weeks' growth, vegetative growth continued and the stromata of individual colonies merged with each other, but there was no sporulation. Apparently aspartic acid will support vegetative growth of the wheat rust once it has started, but will not serve as a source of amino nitrogen for the initiation of colonies.

These results show that chemically-defined media can support luxuriant growth of wheat rust and both growth and sporulation of flax rust without the addition of complex organic substances like peptone or yeast extract. Further work will be required to discover the key to control of sporulation by the wheat rust fungus.

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Anti-receptor antibody and resistance to graft-*versus*-host disease

GRAFT-*versus*-host (G*v*H) disease is a complex syndrome initiated by the reaction of donor lymphoid cells against histocompatibility antigens of the host. Although newborn hybrid animals injected with parental-strain lymphoid cells develop fatal G*v*H disease, adult hybrid animals receiving weight-adjusted doses of such cells develop no clinically evident disease[1,2]. This resistance to G*v*H disease is age-dependent and radiosensitive; resistance develops at 3–4 weeks of age and is abolished by sublethal total-body irradiation[1]. Immunologically specific suppression of G*v*H reactions has been achieved by immunisation of F_1 hybrid host to produce antibody reactive with receptors on donor lymphoid cells for host antigens[3,4].

The G*v*H reactivity of lymphoid cells from the appropriate parental strain was suppressed in F_1 hybrids producing such anti-receptor antibody (ARA)[3,4]. This finding led to the suggestion that ARA could suppress G*v*H reactivity by blocking receptors on aggressor lymphocytes. We have investigated whether the natural resistance of adult F_1 hybrids to G*v*H disease is associated with the production of ARA. We found ARA in rats not developing the disease, while those rendered susceptible to G*v*H by irradiation did not produce ARA.

The radiosensitivity of the processes responsible for the resistance of adult rats to fatal G*v*H disease was confirmed as follows: groups of five adult female Lewis×Brown Norway (LBN) F_1 hybrid rats each either received 400 rad of total-body X irradiation or were left untreated. Twenty-four hours later each group received from 2.7×10^6 to 2×10^8 normal adult female Lewis spleen cells intravenously. Animals were weighed three times weekly and deaths were recorded daily (Fig. 1). All non-irradiated animals receiving Lewis spleen cells survived; none lost weight or had clinically evident disease. All irradiated hybrid rats, however, receiving more than 10^7 Lewis spleen cells died. Weight loss of up to 30% initial body weight preceded death. Control animals receiving 400 rad only had no clinical ill effects. Thus, non-irradiated LBN rats were resistant to fatal G*v*H disease when injected with 100 times more Lewis cells than were required to induce significant and often fatal G*v*H disease in those LBN rats pretreated with 400 rad of X irradiation.

Similar results were obtained when spleen cells from the Brown Norway parental strain were injected in analogous experiments. All unirradiated LBN rats receiving BN spleen cells in doses as high as 2×10^8 survived. All irradiated rats receiving more than 1×10^7 BN spleen cells died of G*v*H disease.

Sera from irradiated and nonirradiated adult hybrid rats receiving parental-strain spleen cells were assayed for ARA by two methods. One method involves a complicated bioassay in which ARA inhibits the formation of a chemotactic factor (product of antigen recognition or PAR) generated when parental-strain lymphoid cells are cultured briefly with hybrid lymphoid cells[5]. The other method involves measurement of the interaction between the specific alloantibody used for immunisation and serum from the immunised hybrid animal[3,6]. Such interaction can be detected by precipitin reactions in gel[3]. In a more sensitive assay, anti-receptor antibody can be detected and quantified by agglutination of sheep erythrocytes to which specific alloantibody has been coupled. ARA produced by immunisation of hybrid rats with alloantiserum and ARA produced by immunisation with parental-strain spleen cells have similar reactivity in all these assays.

To determine whether production of ARA correlated with the resistance of LBN animals to G*v*H disease, samples of serum were obtained from adult female LBN rats that had been injected with normal Lewis spleen cells. Groups of five LBN F_1 hybrid rats were given 400 rad of total-body X irradiation or were left untreated. Twenty-four hours later, 5×10^7 or 1×10^7 Lewis spleen

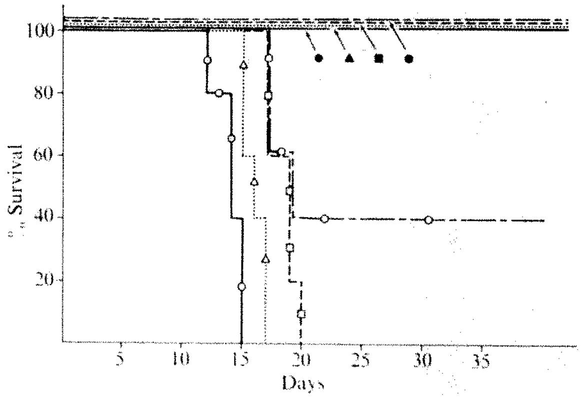

Fig. 1 Survival of adult female LBN F_1 rats after intravenous injection of normal adult female Lewis spleen cells. Groups of 5 F_1 rats each either received 400 rad of total body × radiation (open symbols) or were not irradiated (closed symbols). Twenty-four hours later, the rats were injected intravenously with either 2×10^8 (○ or ●), 5×10^7 (△ or ▲), 1.2×10^7 (□ or ■) or 2.8×10^6 (open and solid hexagons) normal Lewis spleen cells. No F_1 rats receiving 400 rad only (not shown) died.

Table 1 Development of ARA in F_1 hybrid rats receiving parental strain normal Lewis spleen cells

	ARA titre*					
	Days after parental strain spleen cells					
	7	14	21	28†	35	42
5 × 10^7 Lewis spleen cells	0	0	0	64	4	0
	0	0	32	512	128	64
	0	0	16	256	32	0
	0	0	32	64	0	0
	0	0	8	16	0	0
1 × 10^7 Lewis spleen cells	0	0	128	256	Not done	Not done
	0	0	32	64		
	0	0	0	0		
	0	0	8	4		
	0	0	256	64		
400 rad plus 5 × 10^7 Lewis spleen cells	0	0	Dead			
	0	0	Dead			
	0	0	0	Dead		
	0	0	Dead			
	0	0	Dead			
400 rad plus 1 × 10^7 Lewis spleen cells	0	0	0	Dead		
	0	0	Dead			
	0	0	0	Dead		
	0	0	0	0	Dead	
	0	0	0	Dead		

* Reciprocal of the highest serum dilution which causes agglutination of sheep erythrocytes coated with the IgG fraction of Lewis anti-BN serum

† These sera failed to agglutinate SRBCs coated with IgG fractions of Lewis anti-DA serum, Fischer anti-BN serum or BN anti-Lewis serum

cells were injected intravenously and serum samples were obtained at weekly intervals A haemagglutinin assay, involving sheep erythrocytes to which IgG antibody was coupled, was used to measure anti-receptor antibody Lewis anti-BN antibody was purified by ammonium sulphate precipitation and elution from diethylaminoethyl-cellulose with 0 04 M phosphate buffer, *p*H 8 0 After dialysis against saline, the IgG antibody was coupled to sheep erythrocytes by means of chromic chloride[7] Serial dilutions of serum were incubated with sensitised erythrocytes for at least 3 h and the settling pattern was observed (Table 1) Serum samples were also titered with normal sheep erythrocytes, and with sheep erythrocytes to which normal Lewis IgG, Lewis anti-DA IgG, or BN anti-Lewis IgG had been coupled

As observed previously, all irradiated hybrid rats receiving 5 × 10^7 Lewis spleen cells and four or five irradiated rats receiving 1 × 10^7 Lewis spleen cells were dead within four weeks after cell injection ARA could not be detected in any serum sample from the irradiated rats None of the nonirradiated hybrid rats receiving Lewis spleen cells developed G*v*H disease By 4 weeks after injection, 9 of 10 nonirradiated hybrid rats receiving Lewis spleen cells had measurable serum ARA This antibody activity was first noted 3 weeks after injection and in most animals was no longer detectable 3 weeks later The specificity of the ARA reactivity is indicated by the failure of serum samples obtained at 28 d to agglutinate erythrocytes coated with Lewis anti-DA IgG or with BN anti-Lewis IgG The following observations indicate that ARA detected in the serum of hybrid rats receiving parental-strain lymphoid cells is an anti-idiotypic antibody and not a soluble complex of anti-BN antibody and BN antigen formed in antigen excess Such a complex would be expected to agglutinate sheep erythrocytes coated with anti-BN antibody of any idiotype, whereas anti-idiotypic antibody would agglutinate only erythrocytes coated with anti-BN antibody of the appropriate idiotype Fischer anti-BN and Lewis anti-BN IgG fractions possessing equivalent titres of anti-BN haemagglutinin antibody were used to coat sheep erythrocytes The two preparations of coated erythrocytes agglutinated to the same extent with a rabbit anti-rat immunoglobulin serum Serum from hybrid rats obtained 28 d after injection of Lewis spleen cells agglutinated Lewis anti-BN coated cells (Table 1), but did not agglutinate erythrocytes coated with Fischer anti-BN antibody This pattern of agglutination indicates the anti-idiotypic nature of ARA

Thus, ARA is detected in the serum of hybrid rats that do not develop G*v*H disease Irradiation renders hybrid rats susceptible to fatal G*v*H disease, irradiated rats do not produce detectable amounts of ARA Production of ARA may be a part of the radiosensitive process by which hybrid animals resist fatal G*v*H disease If ARA plays such a protective role, lymphoid cells from hybrid rats producing ARA should enable irradiated hybrids to resist fatal G*v*H disease To test this hypothesis, we gave LBN rats 400 rad of total-body X radiation Twenty-four hours later, they received one of the following 10^7 Lewis spleen cells, 10^7 Lewis spleen cells plus 5 × 10^7 normal LBN spleen cells, or 10^7 Lewis spleen cells plus 5 × 10^7 spleen cells from LBN rats that had previously received two injections of Lewis spleen cells All the previously injected LBN rats had ARA in their serum when their spleen cells were taken Irradiated LBN rats given spleen cells from the LBN rats that had resisted fatal G*v*H disease were protected from the lethal effects of injection of parental spleen cells (Fig 2) Normal LBN spleen cells were somewhat less effective in conferring protection to irradiated hybrid rats

Thymus-derived (T) lymphocytes must be present in the responder population[8] to produce G*v*H reactions Recent evidence obtained from G*v*H reactions *in vivo*[9] and mixed lymphocyte cultures *in vitro*[10] indicates that genetic disparity at one or more lymphocyte-defined (LD) loci closely linked to the serologically defined (SD) major histocompatibility complex is essential for a strong proliferative response The generation of cytotoxic effector cells appears to require in addition a disparity at one or more of the SD loci Cyto-

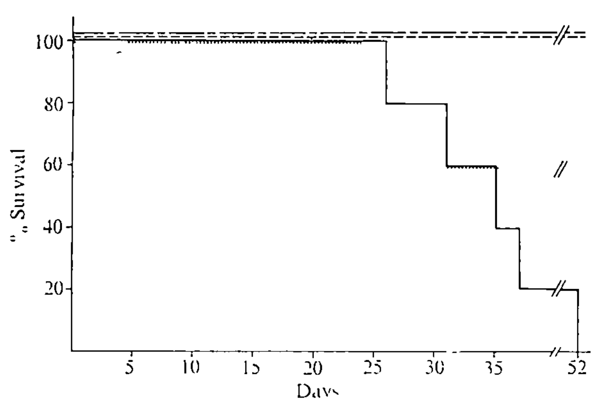

Fig 2 Protection of irradiated LBN F_1 rats from lethal G*v*H disease by adaptive transfer of F_1 spleen cells Irradiated LBN rats receiving 1 × 10^7 normal Lewis spleen cells only (———) all died of G*v*H disease Irradiated LBN rats receiving 1 × 10^7 normal Lewis spleen cells and 5 × 10^7 spleen cells from F_1 rats resistant to G*v*H disease (— — —) suffered no mortality or weight loss The group of irradiated LBN rats receiving 1 × 10^7 normal Lewis spleen cells and 5 × 10^7 normal LBN spleen cells (-------) had an intermediate mortality LBN rats receiving 400 rad of X radiation only (—— ——) all survived No additional mortality occurred in any group during the interval of 52–120 d

toxic effector cells, like the cells responsive to the LD loci, are believed to be T lymphocytes[11]

The generation of effector cells in culture requires DNA synthesis[12] Cultures treated so as to eliminate cells that have undergone DNA synthesis do not yield cytotoxic effector cells[13] Since treatment to eliminate cells that had undergone DNA synthesis could be delayed until placing the cells into the effector cell assay, the cytotoxic effector cells must have replicated during the sensitisation process Thus it seems that proliferation of both LD responsive and SD responsive lymphocytes is required for full cytotoxic expression

Several groups have suggested a model of cellular cooperation involving two populations of T lymphocytes to explain the apparent requirement for both LD and SD responsive T cells Hayry and Andersson[14] suggest that the effector cell responsive to SD antigen requires cooperative interactions of an LD responsive T lymphocyte (and perhaps an adherent cell as well) before it can divide and cause significant target cell lysis

Local GvH reactions may involve predominantly the recognition and proliferative phases of the immune response The syndrome of GvH disease, however, probably includes an additional component of cell-mediated injury initiated by effector lymphocytes[8] If such effector lymphocytes react with the SD antigens of the host through an immunologically specific receptor molecule, then blockade of that receptor may lead to a blunting or inhibition of the killer cell potential of these lymphocytes One immunologically specific receptor molecule able selectively to bind SD antigen is antibody We suggest that the anti-idiotypic antibody demonstrable in the serum of F_1 hybrid rats after injection of parental strain cells may function by blocking or eliminating those parental lymphoid cells which display receptors for the serologically defined histocompatibility antigens of the host

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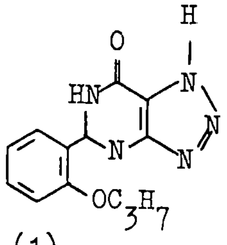
New inhibitor of reagin-mediated anaphylaxis

Methylxanthines, such as theophylline and caffeine, inhibit the antigen-induced release of histamine and of slow reacting substance of anaphylaxis (SRS-A) from passively sensitised human lung and human basophilic leukocytes[1,2] We have shown that these methylxanthines also inhibit a passive cutaneous anaphylactic (PCA) reaction mediated by reaginic antibodies in the rat The methylxanthines are, however, from 20 to 50 times less potent than disodium cromoglycate Because of the relatively low potency of theophylline and because of its other pharmacological actions, notably as a bronchodilator, it is not clear to what extent inhibition of reagin-mediated anaphylaxis may be important in its use for the treatment of allergic bronchial asthma

By progressive modification of the chemical structure of methylxanthines, we have found that certain 2-phenyl-8-azapurin-6-ones[3] possess high and selective anti-allergic activity In particular, 2-*o*-propoxyphenyl-8-azapurin-6-one, M&B 22,948 (1), is from 20 to 50 times more potent than disodium cromoglycate in inhibiting reagin-mediated anaphylaxis in a number of test systems and is also active by oral administration



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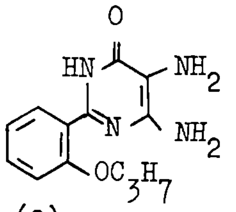

(2)

2-*o*-Propoxyphenyl-8-azapurin-6-one is a white crystalline solid, melting point 241° C (decomp) It is less than 0 1 % w/v soluble in water but readily forms salts, for example the triethanolamine salt has a melting point of 138–139° C The azapurinone was prepared by nitrous acid treatment of the diamine (2) which itself was prepared from *o*-propoxybenzamidine by conventional methods

M&B 22,948 has been compared with disodium cromoglycate in three experimental models involving reagin-mediated anaphylaxis These were first, the inhibition of the allergen induced release of histamine and SRS-A from passively sensitised human lung *in vitro*[4], second, the inhibition of a reagin-mediated PCA reaction in the rat[5] and third, the inhibition of reagin-mediated anaphylactic bronchospasm in the guinea pig In experiments involving aqueous solutions, M&B 22,948 was used as its triethanolamine salt Doses refer to the parent azapurinone

M&B 22,948 was more potent than disodium cromoglycate in inhibiting the anaphylactic release of both histamine and SRS-A from human lung tissue (Fig 1) The effective concentration range for each drug was fairly narrow The optimum concentration for inhibition of mediator release determined in at least seven separate experiments was in the range of 1–3 μg ml^{-1} for M&B 22,948 and 30–100 μg ml^{-1} for disodium cromoglycate

The intravenous dose of M&B 22,948 causing 100% inhibition of the rat PCA reaction was from 35–50 times less than that of disodium cromoglycate Thus, complete inhibition of the rat PCA reaction was consistently obtained with intravenous doses

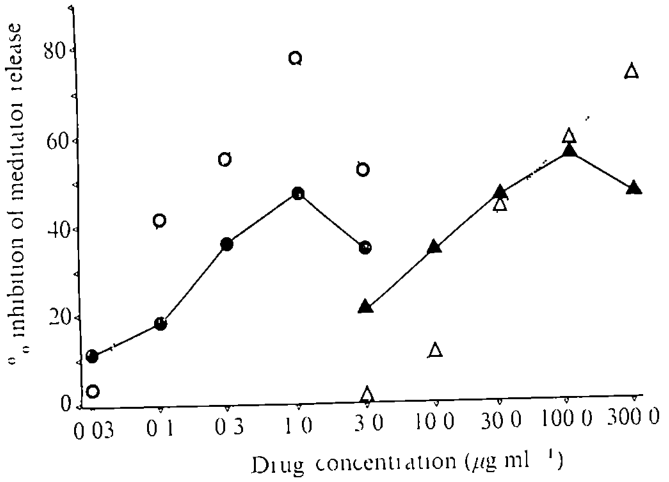

Fig 1 Inhibition of allergen-induced release of histamine and SRS-A from passively sensitised human lung tissue by M&B 22,948 and disodium cromoglycate in two separate experiments Using M&B 22,948 ●, histamine, ○, SRS-A Using disodium cromoglycate ▲, histamine, △, SRS-A Chopped human lung was incubated for 18 h with serum from asthmatic subjects Aliquots were challenged with extracts of *Dermatophagoides farinae* (500 µg ml⁻¹) immediately after the addition of graded concentrations of the test compounds Histamine was assayed fluorometrically SRS-A was assayed on the mepyraminised guinea pig ileum Points refer to single determinations from three pooled samples at each drug concentration

of 0 1 mg kg⁻¹ of M&B 22,948 and 2–5 mg kg⁻¹ of disodium cromoglycate Intravenous theophylline had approximately one-fiftieth the potency of disodium cromoglycate

When administered orally to rats 15 min before allergen challenge, M&B 22,948 was effective in inhibiting the PCA reaction The dose response curve was 'bell shaped' (Fig 2) and complete inhibition of the PCA reaction was rarely obtained Maximal inhibition was obtained with oral doses of between 0 5–2 0 mg kg⁻¹ Disodium cromoglycate showed poor to negligible activity, an oral dose of 500 mg kg⁻¹ produced less than 30% inhibition when administered either 15 or 40 min before allergen challenge Theophylline showed some oral activity with 80% inhibition of the PCA reaction occurring with a dose of 100 mg kg⁻¹

Anaphylactic bronchospasm was produced in the guinea pig passively, systemically sensitised with *Trichinella spiralis* antiserum and premedicated with propranolol (5 mg kg⁻¹, intraperitoneally) The mean preconvulsion time of groups of a minimum of six sensitised guinea pigs exposed to an aerosol of allergen was increased by 19%, 50% and 69% following intravenous doses of 5, 10 and 20 mg kg⁻¹ of M&B 22,948 (Table 1) Disodium cromoglycate (100 mg kg⁻¹ intravenously) produced a protection comparable to that of 10 mg kg⁻¹ of intravenous M&B 22,948 An oral dose of 200 mg kg⁻¹ M&B 22,948 produced a significant protective action Maximum protection was afforded when the animals were challenged 15 min after receiving 200 mg kg⁻¹ M&B 22,948 orally but a significant increase in the preconvulsion time was also seen when challenge was carried out 4 h after drug administration (Table 1) No reduction in the severity of anaphylactic bronchospasm was produced with oral doses of up to 200 mg kg⁻¹ of disodium cromoglycate, administered 15 or 40 min before antigen challenge Theophylline has not been studied in these tests

Table 1 Inhibition of reagin-mediated anaphylactic bronchospasm in the guinea pig by M&B 22,948 administered intravenously, orally or by the inhalation of an aqueous aerosol

Route of drug administration	Dose (mg kg⁻¹)	Time (min) between dose and bronchial allergen challenge	Mean % protection
Intravenous	5	1	19 (4)†
	10	1	50 (4)
	20	1	69 (4)
Oral	200	15	62 (4)
		40	54 (2)
		60	48 (6)
		120	43 (3)
		240	42 (3)
Inhalation	50 (mg ml⁻¹)	1	34(11)

The % protection was 100 [1−(*C*/*T*)] where *C* and *T* were the preconvulsion times of control and treated groups

*Concentration of aerosol generating solution Animals exposed to aerosol for 2 min

†Figures in parentheses refer to the number of experiments

M&B 22,948 antagonised some of the pharmacological effects of the mediators of anaphylaxis Thus, concentrations of M&B 22,948 of 1–30 µg ml⁻¹, which inhibit the anaphylactic release of mediators from human lung tissue, also antagonised the spasmogenic effects of histamine, SRS-A and $PGF_{2\alpha}$ on isolated human bronchial muscle In the rat, intravenous doses of 10–25 mg kg⁻¹ of M&B 22,948 were required to reduce the increase in capillary permeability produced by intradermal histamine and 5-HT These dose levels are considerably higher than that of M&B 22,948 required to inhibit the reagin-mediated PCA reaction (0 1 mg kg⁻¹ intravenously)

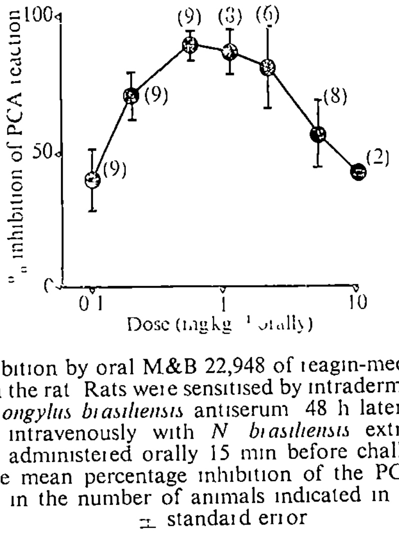

Fig 2 Inhibition by oral M&B 22,948 of reagin-mediated PCA reactions in the rat Rats were sensitised by intradermal injection of *Nippostrongylus brasiliensis* antiserum 48 h later they were challenged intravenously with *N brasiliensis* extract M&B 22,948 was administered orally 15 min before challenge Each point is the mean percentage inhibition of the PCA reaction determined in the number of animals indicated in parentheses ± standard error

The concentrations of M&B 22,948 (µg ml⁻¹) required to cause a 50% reduction in the heights of the contractions of superfused guinea pig ileum produced by various agonists were as follows histamine, 32, SRS-A, 7, acetylcholine, 1, PGE_2, 15, $PGF_{2\alpha}$, 2 5, 5-HT, 6 6 All these values were within the range of concentrations which inhibit the reagin-mediated anaphylactic release of histamine and SRS-A from human lung tissue As the histamine released from the human lung tissue was determined fluorimetrically, the antihistamine activity of M&B 22,948 would not have interfered in the assays M&B 22,948 showed no significant activity, either intravenously or orally, in protecting conscious guinea pigs from histamine-induced bronchospasm Similarly, in experiments carried out in the anaesthetised guinea pig, using either the Konzett–Rossler or the Dixon–Brodie method of recording bronchial tone, no indication was obtained of bronchodilator action or of a specific antihistamine or anti-5-HT action of M&B 22,948

Certain 2-phenyl-8-azapurin-6-ones, and in particular M&B 22,948, have been shown to have high activity in inhibiting the release of the pharmacological mediators of anaphylaxis from isolated human lung tissue *in vitro*, in a reagin-mediated PCA reaction in the rat, and in reagin-mediated anaphylactic bronchospasm in the guinea pig The potency of M&B 22,948 was up to 50 times that of disodium cromoglycate, depending on the test system and the method of drug administration M&B 22,948 may be of interest in the prophylactic treatment of allergic bronchial asthma Since this work was carried out, we have learnt of the oral activity of an oxoxanthene derivative (AH 7725) in inhibiting an allergen induced immediate-type asthmatic response in man[6]

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Significance of immunofluorescent staining of lymphocytes with antisera to IgM immunoglobulins

IMMUNOLOGICAL reactions depend for their completeness on at least two different populations of lymphocytes, one derived from the bone marrow and the other from the thymus (B and T cells) B lymphocytes, precursors of the antibody-forming cells of vertebrates, are recognised *in vitro* by two characteristic reactions surface fluorescence with antisera to immunoglobulins[1] and rosette formation with red cells sensitised with some components of complement[2] Since the cells that show surface immunofluorescence are retained on columns of beads coated with antibodies to immunoglobulins[3], surface fluorescence is generally interpreted to mean that immunoglobulins are present on the surface of B lymphocytes[4]

Various means have been devised to prove this point primarily by using high resolution optical devices[5] or specific antisera[6] Although antisera to IgM immunoglobulins bind specifically to structures on the surface of lymphocytes, in only two cases to our knowledge have IgM immunoglobulins been separated from lymphocytes[7,8] and in such small quantities that the authors had to rely on measurements of radioactivity of subcellular fragments obtained by specific coprecipitations to support their assertions It is generally believed as a result of these and other experiments, that lymphocytes indeed bear immunoglobulins and in particular IgM immunoglobulin, on their surface We were therefore surprised to find that it was possible to

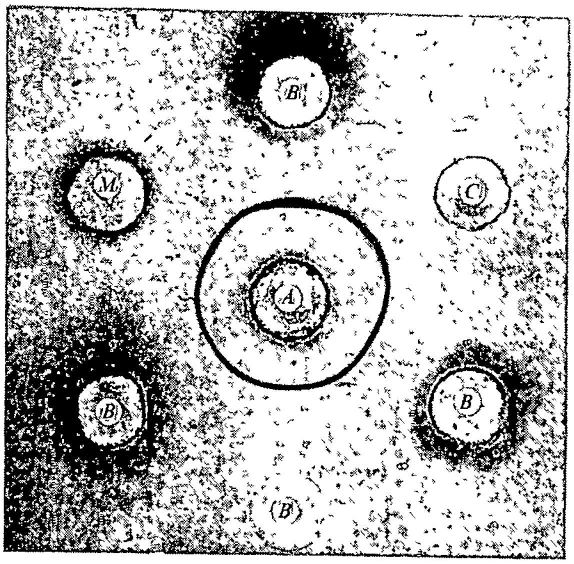

Fig. 1 Double diffusion in agar of IgM immunoglobulin (*A*) with anti-CHO (*C*), anti-μ (*M*), and anti-IgM (*B*) The reaction shows complete immunological identity of the three antisera with IgM immunoglobulin Purified IgM immunoglobulin was prepared by gel filtration of 1 4 l of normal human serum on columns of Biogel P300 resin The fraction eluting in the exclusion volume of the resin was concentrated over Ficoll and applied to a column of ethanolysed cellulose in a Tris borate buffer of *p*H 9 4 The colloids were electrophoresed at a potential of 240 V for 20 h at 4° C and eluted from the column at a rate of 10 ml h^{-1} The fractions reacting with an antiserum to human IgM immunoglobulin were pooled, dialysed against 0 15 M NaCl, and re-electrophoresed α-macroglobulin could be separated from the IgM in this way Fractions containing IgM immunoglobulin were chromatographed twice on Sephadex G200 resin to a single homogeneous chromatographic peak (0 330 g) By electrophoresis, sedimentation and precipitation with an antiserum to human IgM immunoglobulin over 98% of the protein contained in this fraction was shown to be IgM immunoglobulin At no time during this preparation were the soluted containing IgM immunoglobulin frozen The purified IgM was digested first with trypsin (1% w/w) at *p*H 8 0 in 0 65 M Tris HCl containing 0 01 M $CaCl_2$ for 24 h at 37° C, followed by pronase (1% w/w) under the same conditions for an additional 24 h at 37° C The digested protein was dialysed against distilled water The dialysate was concentrated *in vacuo* and applied to a column of Sephadex G25 (fine) resin (0 8 × 200 cm) in 1% ammonium bicarbonate Materials eluting from the Sephadex resin were analysed for carbohydrate by the Mohlish reaction and the Mohlish-positive materials were rechromatographed on Sephadex G25 to a single symmetrical peak The materials within this peak were concentrated *in vacuo* and separated by paper chromatography (butanol–ethanol–acetic acid–water 8 2 1 3) Five oligosaccharides were separated in this way The anti-CHO antiserum (*C* above) is specific for these five oligopeptides

quantify IgG separable from human B cells[9] and that human B lymphocytes could be induced to synthesise IgG *in vitro*[10], but we were unable either to quantify IgM or to show synthesis of IgM in these cells even though they fluoresced with antisera to IgM immunoglobulin Some infants with severe combined immunodeficiency possess in their serum a monoclonal IgG, we have shown that lymphocytes from the serum of one of these children could be induced to synthesise *in vitro* the monoclonal IgG that was in the child's serum[11] Yet all of his lymphocytes showed surface fluorescence with an antiserum to IgM We decided to study this apparent discrepancy We wish to present evidence which will show that surface immunofluorescence of lymphocytes with antisera to IgM is not caused by the presence of IgM on the surface of these lymphocytes

Human IgM was prepared by conventional physico-chemical procedures from normal human serum Over

94% of the protein in this preparation sedimented in the centrifuge as a single boundary with a sedimentation coefficient of 19S It precipitated quantitatively with an antiserum to human IgM

Proteolysis of IgM with trypsin and pronase has been shown to degrade extensively both μ and L chains of IgM[12] Five glycopeptides containing a minimum of 5 and a maximum of 13 sugar residues each, together with six to nine amino acids, represent the only material separable in the exclusion volume of a Sephadex G25 resin when a trypsin-pronase digest of IgM is analysed Each oligopeptide contains N-acetyl glucosamine as the reducing carbohydrate end group, and a fucose (oligopeptide 1 and 2) or a mannose (oligopeptide 3 to 5) in each case bound to an aspartic acid of the polypeptide chain, representing the non-reducing carbohydrate ends These five glycopeptides comprise all the carbohydrate of the μ chain[13] Purified IgM was digested with trypsin followed by pronase The glycopeptides obtained after hydrolysis were separated on Sephadex into Mohlish-positive materials that on paper chromatography (butanol–ethanol–acetic acid–water 8 2 1 3) were shown to be composed of five chemical entities each containing carbohydrate residues We obtained 10 mg of these glycopeptides (dry weight)

An immunoadsorbent resin was prepared by a modification of the method of Axen and Ernback[14] To assure that the carbohydrate moiety of the glycopeptides would bind to the immunoadsorbent, Sepharose 4B activated with CNBr was coupled to L-lysine CNBr-activated IgM glycopeptides were in turn, coupled to the Sepharose–lysine adduct Over 84% of the carbohydrate was bound to the Sepharose in these conditions

IgG globulin was separated (DEAE cellulose) from a rabbit antiserum to normal human IgM globulins The antiserum was rendered monospecific for the μ chains of IgM by passage through immunoadsorbents made by coupling human IgG and IgA immunoglobulins and BenceJones proteins to Sepharose 4B This rabbit IgG globulin (1 g) was incubated at 37° C for 2 h with the immunoabsorbent containing the glycopeptides of IgM in phosphate-buffered saline (PBS) at *p*H 7 2 The unbound globulin was eluted at room temperature, and the absorbent washed with PBS Bound antibodies were eluted with a glycine/HCl buffer, *p*H 2 0 (0 2 M) After five adsorption cycles, no antibodies remained bound to the immunoadsorbent A total of 3% of the rabbit IgG fraction was eluted as specific antibody to the glycopeptides (anti-CHO) The remainder was designated anti-μ even though antibodies to μ determinants of IgM were not specifically isolated Both fractions (anti-CHO and anti-μ) reacted with human IgM as shown in Fig 1 Both antisera gave reactions of complete fusion against IgM Immunodiffusion in agarose (1 5%) rather than agar did not alter the pattern of precipitation Neither the anti-CHO nor the anti-μ precipitated in agar with the glycopeptides isolated from IgM as described above, showing that the glycopeptide fraction did not contain large μ-chain fragments that could react with either the fractionated or non-fractionated antiserum The anti-CHO and anti-μ antibodies had sedimentation coefficients of 6 8 and 6 6, respectively, indicating that neither antibody contained aggregated γ globulin Fluorescent antisera were prepared by dialysing the proteins against fluorescein isothiocyanate buffered at *p*H 9 0 (ref 15) Fluorescein-labelled anti-IgM, anti-CHO, and anti-μ were reacted with B cells The results are summarised in Table 1

Table 1 Percentage of cells staining with fluorescein-labelled antisera to human IgG and Igm Immunoglobulins and the glycopeptides (CHO) or polypeptide chains (μ) of IgM

Cell type	Pre-incubation of Cells with antisera to	Pre-incubation of Antisera with antigen	Fluorescein-labelled antibody (% of cells staining) IgM	CHO	μ	IgG
B1*			94(91–99)	95(91–99)	<2	<2
B1	CHO		20(12–32)	20(12–32)		
B1	IgM		22(10–33)	16(8–26)		
B1	μ		91(90–96)	94(90–98)		
B2†			55(45–70)	56(50–62)	<2	20
B2	CHO		19(12–27)	15(10–22)		
B2	IgM		17(9–23)	14(8–21)		
B2	μ		54(42–66)	56(46–69)		
BM‡			40§	10	40§	
BM		IgM	10	10	<2	
BM		CHO	40	<2	40	

Fluorescent antisera were prepared by dialysing the protein to be labelled (1 ml of a 1% solution) against 300 ml of a solution containing fluorescein isothiocyanate (0 5 mg ml^{-1}) buffered at *p*H 9 0 for 72 h in the dark Labelled antisera were dialysed extensively against PBS and stored in the dark 0 1 ml of fluorescein-conjugated antisera was added to an equal volume of a suspension containing 2×10^6 cells ml^{-1} in medium 199 supplemented with 5% foetal calf serum and 10^{-2} mol l^{-1} sodium azide Cells were incubated for 45 min in the cold, washed three times in medium 199, and transferred to a glass slide in a drop of glycerol PBS (9 1) Slides were examined, and the percentage of fluorescent cells recorded

* Six determinations

† Four determinations

‡ Bone marrow from a patient with Waldenstrom's macroglobulinemia (one determination)

§ Cytoplasmic staining (all others were surface stained)

B cells were separated from peripheral blood lymphocytes or from tonsillar lymphocytes by density centrifugation on gradients of bovine serum albumin[16] Two different populations of B cells were obtained from the gradients, both of which possessed a complement receptor indicated by rosette formation of these cells with EAC1423 One cell population comprised large cells that sedimented in the upper region of the gradient (17–21% albumin, B1) These could not be induced to form IgG *in vitro* The second cell population comprised small cells with a high density which sedimented toward the bottom of the gradient (29–31% albumin, B2), and these could be induced to form IgG immunoglobulins *in vitro* Over 95% of B1 cells reacted with fluorescein-labelled anti-IgM or anti-CHO, but only 55% of B2 cells did so Less than 2% of either B1 or B2 cells reacted with anti-μ For comparison, less than 2% of B1 and 20% of B2 cells reacted with fluoresceinated anti-IgG Upwards of 80% of the fluorescence of B1 cells and 70% of B2 could be inhibited when the cells were preincubated with unlabelled anti-CHO or anti-IgM, but the fluorescence remained unaltered when either cell was preincubated with anti-μ The specificity of the antisera was tested by incubating the bone marrow of a patient with Waldenstrom macroglobulinemia with fluorescein-labelled anti-IgM, anti-μ and anti-CHO

Two types of fluorescent pattern were visible, cytoplasmic fluorescence and surface fluorescence The cytoplasmic fluorescence of the plasmacytoid cells of the marrow synthesising IgM was revealed both with the anti-IgM and anti-μ antisera This experiment (positive control for anti-μ) indicated that the anti-μ antiserum had activity against the μ chain determinants of IgM This activity could be inhibited by preincubating the antiserum with IgM but not with the glycopeptides of IgM, showing specificity of this antiserum for the polypeptide chains of IgM A small amount of B1 cells were present in the marrow (10%), and presumably it was these cells that reacted with the antiserum to IgM or to the glycopeptides in a pattern showing surface fluorescence This activity could be inhibited by preincubating the antiserum with the glycopeptides but not with IgM showing specificity of the antiserum for the glycopeptides of IgM but not for the polypeptide chains of IgM Comparable results were obtained when the following experiments were done Anti-IgM antiserum (5 ml) was adsorbed 10 times with 10^7 B1 cells each time at 37° C for 1 h The adsorbed antiserum showed unimpaired reactivity with IgM by double diffusion in agar It failed, however, when preincubated with either B1 or B2 cells to inhibit the fluorescence of the anti-CHO antiserum with both B1 and B2 cell populations

Immunoglobulins are glycoproteins Over 10% of the mass of IgM is carbohydrate so that antisera to IgM should contain antibodies directed to the carbohydrate, as well as to the polypeptides of the immunoglobulin chains The data presented here show that immunofluorescence of B lymphocytes with antisera to IgM is not caused by IgM immunoglobulin present on the surface of B lymphocytes It is rather caused by interaction of antisera to IgM immunoglobulin with carbohydrate on the surface of the lymphocyte This carbohydrate must be cross reactive and, therefore, structurally similar to the carbohydrate of IgM immunoglobulin

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Cellular immune response to a drug-treated L5178Y lymphoma subline

TUMOUR-specific transplantation antigens (TSTA), have been detected on a wide range of experimental tumour cells and elicit weak immunogenic activity As a rule, little immune response of the host to autocthonous tumours or to implanted syngeneic tumours has been detected Furthermore, immunological techniques, such as active immunisation, passive transfer of antibodies or adoptive transfer of immune lymphocytes, have not been entirely satisfactory Attempts have also been made to increase the immunogenic properties of tumour cells by *in vitro* enzymatic treatments[1] or *in vitro* coating of antigenic determinants on cancer cells[2]

Some studies have shown that it is possible to increase the immunogenicity of tumour cells[3]. In our laboratory immunogenic L1210 sublines were induced by prolonged *in vivo* treatment with the drug 5-(3,3-dimethyl-triazeno)imidazole-4 carboxamide (DIC)[4] Mice compatible with the untreated L1210 leukaemia were resistant to a heavy challenge of L1210/DIC cells, whereas host resistance could be abrogated by immunodepression The alteration of antigenicity acquired by L1210 DIC-treated cells (L1210/DIC) was found to be heritable[4,5]

We present evidence that a subline of L5178Y tumour, transformed by DIC *in vivo* (L5178Y/DIC) (manuscript in preparation) implanted into mice compatible with the parental leukaemia, stimulated a specific cell-mediated cytotoxic response

L5178Y/DIC was maintained by serial intraperitoneal (i p) passages in immunosuppressed 200 mg kg^{-1} (cyclophosphamide given intraperitoneally 24 h before tumour challenge) hybrid CDF_1 mice Other tumour lines used in this study were maintained by serial i p passages in compatible inbred mice

The cellular immune response was evaluated by the *in vitro* ^{51}Cr release assay[6], modified by Canty[7]

A sample of 10^7 spleen cells (attackers) from normal (control) or immune mice were suspended in a plastic Petri dish (Falcon, 35 × 10 mm) in 1 ml Medium 199 (DIFCO) supplemented with 10% heat inactivated foetal calf serum (GIBCO) and incubated for 4 h with 5×10^4 (50 µl) ^{51}Cr-labelled cells (targets) on a rocking platform at 37° C in a moist atmosphere of 10% CO_2–90% air Radioactivity released in the supernatant by labelled target cells was counted in an automatic scintillation counter

In each experiment quadruplicated samples were used for each experimental group and the data reported are from one out of three similar experiments The percentage cytotoxicity for each group was calculated as follows

$$\frac{\text{c p m } ^{51}\text{Cr released from incubated cells}}{\text{c p m } ^{51}\text{Cr released from frozen–thawed cells (4 times)}} \times 100$$

Experimental results were expressed as (% cytotoxicity in experimental samples)—(cytotoxicity in control samples) The ^{51}Cr release in control samples was 12 ± 3%

Spleen cells from mice immune to L5178Y/DIC tumour cells were highly cytotoxic *in vitro* to ^{51}Cr-labelled L5178/DIC cells Parental radiolabelled L5178Y cells and other tumour and thymus cells syngeneic or allogeneic with CDF_1 mice, did not release the isotope after incubation with anti-L5178Y/DIC attacker cells The specific cell-mediated ^{51}Cr release indicates

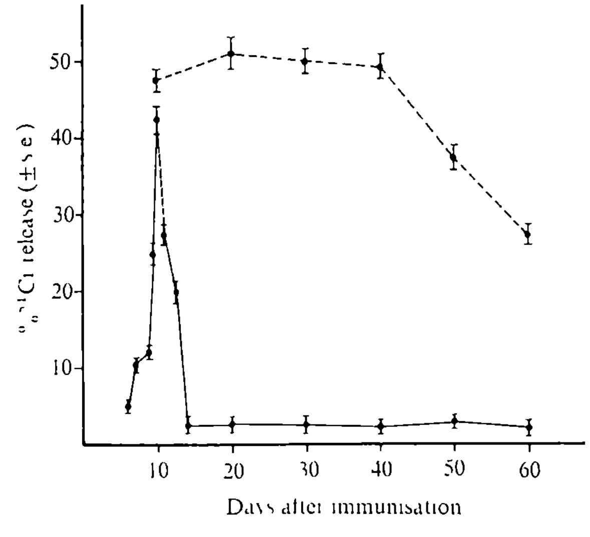

Fig 1 Kinetics of immune spleen cell activity —, Direct assay, ---, assay 24 h after incubation Attacker cells were from CDF_1 mice immunised with 10^7 L5178Y/DIC viable cells injected i p and assayed against ^{51}Cr-labelled L5178Y/DIC cells In these and the following assays the ratio of attacker cells to labelled target cells was 200 1

Table 1 Cytotoxic activity of spleen cells immunised against allogeneic antigens

Attacker cells	Target cells	^{51}Cr release (±s e)
S_{EL_4}	EL$_4$	14 1 (0 4)
	L5178Y	3 4 (0 4)
	L5178Y/DIC	2 8 (0 6)
$S_{E♂G_2}$	E♂G$_2$	16 6 (0 8)
	L5178Y	3 4 (1)
	L5178Y/DIC	−2 (0 9)
S_{C_3H}	C$_3$H	42 8 (0 7)
	L5178Y	3 3 (0 9)
	L5178Y/DIC	−1 8 (0 9)
$S_{C57BL/10\ Sc\ Cr}$	C57BL10/Sc Cr	12 4 (1)
	L5178Y	2 (0 6)
	L5178Y/DIC	3 5 (0 8)

Attacker cells (S) were spleen cells from CFD$_1$ mice 10 d after immunisation with an i p inoculum of 10^7 tumour cells (EL$_4$–E♂G$_2$) or a Sc implant of 1 mm^3 skin tissue pieces (C$_3$H—C57BL/10 Sc Ci) L5178Y and L1210 were from Microbiological Ass Bethesda EL4 and E♂G2 were obtained from Dr G Della Porta, National Cancer Institution Milan Moloney Sarcoma (MSV) and Gross leukaemia (GLV) were virus-induced by Dr A Di Marco, National Cancer Institution, Milan

that DIC treatment can induce transplantation antigen(s) on L5178Y/DIC cells, not detectable on the original L5178Y lymphoma and on other tumour, or normal thymus cells

The cytolytic activity of spleen cells from CDF$_1$ mice assayed at different days after administration of the immunising inoculum is shown in Fig 1 The activity reached a peak at 10 d after tumour cell inoculation and could not be detected after 13 d 24 h *in vitro* incubation fully reactivated the cytotoxic activity and it was still evident at 60 days following immunisation The immune cell reactivity, its disappearance a few days after the peak was reached as the result of a block by some still unknown factor and its reactivation by 24 h incubation, agree with previous observations involving the ^{51}Cr release assay technique[8]

Immune spleen cells from CDF$_1$ mice sensitised to allogeneic antigens were cytotoxic to ^{51}Cr-labelled tumour cells bearing the corresponding alloantigens (Table 1) The spleen cells failed to release the radiolabel from either the original L5178Y or the L5178Y/DIC suggesting that the cells of the subline had not acquired some nonspecific immunosensitivity Evidently neither L5178Y or L5178Y/DIC associated antigens can react with H—2^b or H—2^k associated antigens

The specificity of L5178Y/DIC antigen(s) was determined by using an assay for inhibition of cytotoxicity involving addition of unlabelled cells Addition of unlabelled cells to the mixture of immune lymphocytes and labelled L5178Y/DIC target cells would result in inhibition of ^{51}Cr release if the unlabelled cells shared some common antigen(s) with L5178/DIC cells Only unlabelled L5178Y/DIC cells elicited marked inhibitory activity (Table 2) All other normal or tumour cells added, including the parental L5178Y cells, did not modify ^{51}Cr release significantly The failure to detect L5178Y/DIC antigens on foreign cells provides strong evidence of the specificity of L5178Y/DIC induced antigen(s)

Table 2 Inhibition of immune spleen cell-mediated cytotoxicity by addition of unlabelled cells

Added inhibitor cells	% Reduction in lysis*	
	5 × 10^5	10^5
L5178Y/DIC	68 (7 8/24 9)	39 (15 3/24 9)
L5178Y	6 (15 6/16 6)	−6 (17 6/16 6)
L1210	8 (22 9/24 9)	4 (24/24 9)
E♂G$_2$	−2 (39/38)	−5 (40/38)
EL$_4$	13 (22 7/26 4)	13 (22 7/26 4)
MSV	12 (14 3/16 1)	4 (15 4/16 1)
GLV	16 (13 5/16 1)	0 (16 1/16 1)
Spleen DBA/2 Cr	−11 (21/18 8)	0 (18 8/18 8)
CDF$_1$	0 (18 8/18 8)	2 (19 1/18 8)
BALB/c	8 (23 5/25 5)	3 (24 7/25 5)
C57BL/10 ScCr	4 (18/18,8)	8 (17 2/18 8)
C$_3$H	8 (23 5/25 5)	4 (24 5/25 5)

Attacker cells were from CDF$_1$ mice i p challenged with 10^7 L5178Y/DIC viable cells and assayed after 10 d against ^{51}Cr labelled L5178Y/DIC cells

* $\frac{\%\ \text{lysis with inhibitor cells}}{\%\ \text{lysis without inhibitors}}$

Immune cells were assayed immediately or 4 h after X-ray treatments The activity of the attacker cells was only partially reduced even by heavy doses of X rays (2 00–4 00 r), in accordance with the known resistance of cytolytic cells (immune lymphocytes and macrophages) to X irradiation (Table 3)[9]

Our studies indicate that L5178Y/DIC lymphoid cells carry new antigen(s) not detectable on parental cells The mechanism, by which DIC alters the immunological properties of L1210 and L5178Y lymphomas is unknown The drug-induced antigen(s) were not found on normal lymphocytes or on cancer cells But the strong transplantation antigen(s) seems to be a permanent genetic feature, as the L5178Y/DIC cells maintained the acquired antigen(s) even when the drug treatment was discontinued over a long period

Table 3 Cytotoxic activity of X-irradiated immunised spleen cells

X-ray dose	% lysis (± s e)	% Reduction in lysis relative to non-irradiated cells
Immediate assay		
—	20 3 (0 9)	—
500 r	19 4 (0 8)	5
1,000 r	18 5 (0 7)	9
2,000 r	12 4 (0 5)	39
4,000 r	8 1 (1 1)	61
After 4 h assay		
—	18 (0 7)	—
500 r	17 1 (0 9)	5
1,000 r	17 1 (1)	5
2,000 r	14 3 (0 6)	21
4,000 r	10 3 (0 8)	43

Attacker cells were from CDF$_1$ mice challenged i p with 10^7 L5178Y/DIC viable cells and assayed after 10 d against ^{51}Cr-labelled L5178Y/DIC cells Attacker cells were X irradiated (Securix Compact -CGR, 200 kV, 12 mA, filter Cu 0 5 mm, Al 0 5 mm, 23 cm distance, dose rate 98 2 r min^{-1}) at 4° C and the activity tested immediately or after 4 h incubation at 4° C

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A lymphocyte-inhibiting factor isolated from normal human liver

WHILE we were looking for cell-mediated immunoreactions in patients with active chronic hepatitis (ACH), non-reproducible results with the leukocyte migration inhibition test[1], using

Table 1 Dose-dependent effect of inhibitor on PHA- and MLC-stimulated human lymphocytes and HeLa cells

Inhibitor (μg/0 2 ml)	Lymphocytes PHA (^{3}H-Thymidine Mikro) (Ly-control) (c p m)	Inhibitor (μg/0 2 ml)	Lymphocytes MLC (^{3}H-Thymidine Mikro) (Ly-control) (c p m)	Inhibitor (μg/0 2 ml)	HeLa ^{3}H-Thymidine (c p m)	HeLa ^{3}H-Thymidine (c p m)
0	8,442 (59)	0	7,319 (312)	0	77,000	42,000
—	—	8	5,116	6 2	78,000	33,000
12	2,923	12 5	1,590	12 5	80,200	34,000
16	102	—	—	—	—	—
25	26	25	288	25	61,300	27,000
50	39	—	n d	50	n d	22,000
100	28	—	n d	100	n d	12,000

different preparations of liver antigen from human liver, led us to attempt to prepare a better defined antigen from human liver—already proposed[2] for this test system Besides a liver specific protein as previously described[11], our preparation yielded a soluble fraction, which inhibited the peripheral leukocytes of patients with active chronic hepatitis, but also inhibited the leukocyte migration of controls The migration inhibition in these cases was therefore not due to a cell-mediated immune reaction against liver antigen, but was caused by a previously unknown inhibiting substance, extracted from human liver Here we report some properties of this inhibitor

The inhibitor was isolated from the soluble supernatant after homogenising and centrifugation of normal human liver at 100,000*g* for 90 min in Hanks solution, *p*H 7 2 The supernatant was transferred to a Sephadex G-100 column, equilibrated with 0 01 M Tris-HCl buffer, *p*H 8 0 The inhibiting factor was eluted in the second (II) and third (III) fraction Rechromatography of these active fractions yielded a homogeneous peak, but no pure substance An ion exchange chromatography procedure was therefore added, and we obtained a pure substance with inhibiting properties

This inhibitor is a protein The molecular weight, estimated by gel filtration, is about 65×10^3–8×10^3 The electrophoretic mobility is slow, similar to the β-γ-globulins

The functional properties of this protein are characterised by a potent inhibition of lymphocyte transformation after stimulation by phytohaemagglutin (PHA), purified protein derivative (PPD) and by cellular antigens or transplantation antigens (mixed lymphocyte culture (MLC)) (Fig 1) Besides, the metabolism of *in vivo* activated lymphocytes[3,4] and the migration of the macrophages are inhibited also After PHA stimulation, most of all the *de novo* synthesis of RNA is inhibited

The specificity of lymphocyte metabolism inhibition seems to be very high Doses which cause a 100% inhibition of lymphocyte's metabolism lead only to a very slight reduction of the metabolic rate of HeLa cells (Table 1) On the other hand, no species specificity of the inhibitor could be found, since a preparation from rabbit liver has about 40% of the inhibiting potency on human lymphocytes compared with inhibitor prepared from human liver

The inhibitor reported here is not identical with the immunosuppressive α globulins from human serum[6,7], because the inhibitor from human liver has an electrophoretic mobility of a β or γ globulin We have isolated this inhibitor from the pooled serum of normal people also (by gel-filtration) but the inhibiting strength of this preparation was only 10% of that prepared from liver We therefore suppose that the inhibitor is released from the liver to the serum

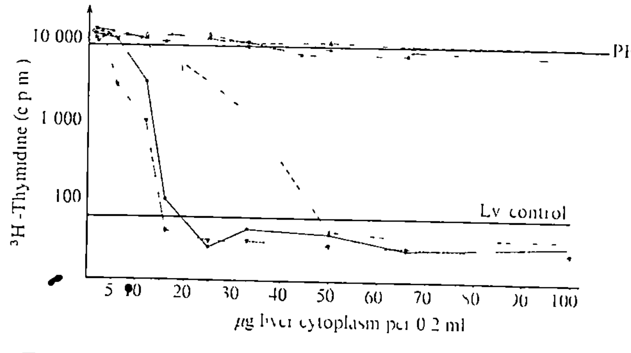

Fig 1 Dose-dependent inhibition of PHA-stimulated lymphocytes by fractions (Sephadex G100) from soluble liver cytoplasm ○ - - - ○, Fraction I, X — X, fraction II ▽ - - - ▽, fraction III, X —— X, fraction IV, △ — △, fraction V, ●—●, liver cytoplasm

Whether or not the prolonged survival time of skin allografts with simultaneous liver transplants on *Diemictylus viridescens*[7] was due to the immunosuppressive effect of the lymphocyte inhibitor from liver is unknown The clear cut time dependency of liver implantation and immunosuppressive effect and the survival of the skin allograft does not exclude this possibility

The inhibitor described here has some similarity with the inhibitor of protein synthesis in polyribosomes, reported by von der Decken[8] The fact that the polyribosomes were prepared from rat liver, however, makes it unlikely that the inhibitor of protein synthesis in polyribosomes and our inhibitor are identical or similar The inhibitor reported here seems to be lymphocyte specific

The inhibitor is not a chalone[9], because chalones are defined as non species-specific inhibitors, present in and produced by the tissue, the proliferation of which they specifically inhibit[10]

The physiological significance of this inhibitor remains to be elucidated, especially the mode of action and the question whether or not this substance has something to do with regulation or repair mechanisms Studies on the inhibitory effect on T or B lymphocytes are in progress

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Two types of resistance to polyene antibiotics in *Candida albicans*

STUDIES reported during the past 15 years have led to the widely accepted conclusion that polyene antibiotics function

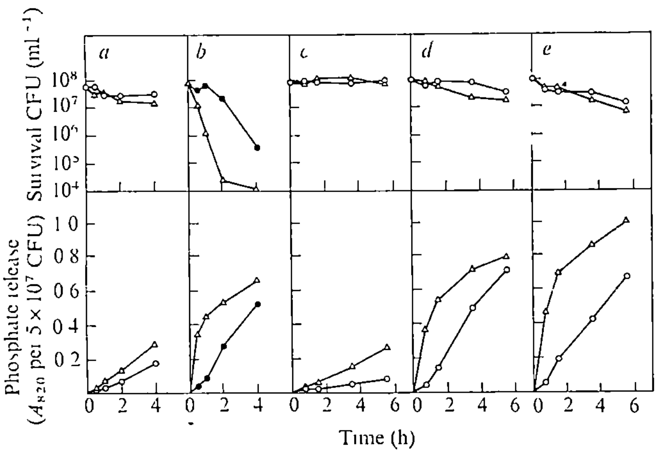

Fig 1 The effect of polyene on the viability (CFU ml^{-1}) and on the release of phosphate ($A_{820\,nm}$ per 5 × 10^{7} CFU) from mutants A1 (*a*), A2 (*b*), A3 (*c*), A4 (*d*) and A7 (*e*) at room temperature Only exponentially growing cells were suspended in saline After incubation with polyene for the periods shown, viability was determined by plate-counting and the release of phosphate was quantitated colorimetrically[21] on the supernatant fluid after centrifugation The amount of release in the blank control samples remained negligible ●, 10 μg amphotericin B ml^{-1}, ○, 40 μg amphotericin B ml^{-1}, △, 100 μg nystatin ml^{-1}

by binding with the sterol molecules in susceptible cells to cause permeability change and eventual death of the cell[1-3] In recent reports[4-6] based on liposomal model membrane studies, it has been suggested that susceptibility to polyene antibiotics is determined by organisation rather than by a single component of the membrane, namely sterol Here, through study of polyene-resistant mutants, we further propose that, at least in some cases, permeability alteration may not be the specific critical event in the fungicidal action of polyene antibiotics

Five mutagen-induced mutants were derived from a clinical isolate of *Candida albicans* (E139-A, wild type) as described by Hamilton-Miller[7] Their levels of resistance vary with inoculum size and with the medium (Table 1) For example, mutant A2 grew in 100 μg ml^{-1} nystatin on solid medium but was killed in saline Compared with E139-A, the mutants grew more slowly, responded differently in the Liebermann–Burchard colorimetric reaction (Table 1) and, in accordance with previous reports[8,9], contained proportionally more sterol (Table 2) In other laboratories, differences in the sterol pattern of polyene-resistant mutants have been observed[8-13], and the major sterols have been characterised completely in one set of mutants[14] Polyene resistance was attributed to altered patterns of sterols In several studies[10,11,13], decrease in ergosterol content has been singled out as the direct cause of polyene resistance For comparison, the ultraviolet absorption spectra of the non-saponifiable fraction extracted from E139-A and our polyene-resistant mutants were examined[10] In all cases, 5,7-diene sterols (for example, ergosterol and 24(28)-dehydroergosterol) were detected but not as major components This was confirmed by analysis of the sterol composition with thin-layer chromatography (Table 3) A similar situation has been reported in a strain of *Saccharomyces cerevisiae* in which ergosterol constituted only 10% of the total sterol[15]

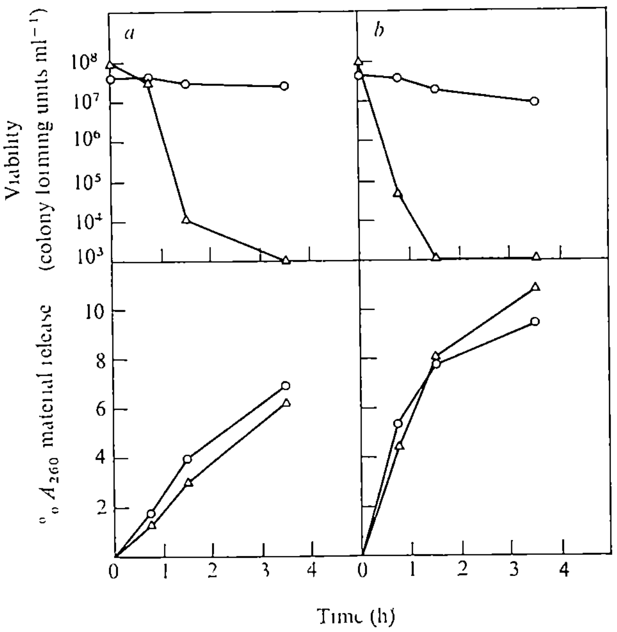

Fig 2 The effect of polyene on the viability (CFU ml^{-1}) and on the release of ultraviolet-absorbing substances (260 nm) from the wild type E139-A (△) and from mutant strain A4 (○) Exponentially growing cells were used In (*a*) amphotericin B was present at 20 μg ml^{-1}, in (*b*) nystatin was present at 100 μg ml^{-1} Experimental conditions were identical to those described for Fig 1 The maximal extractable ultraviolet-absorbing material from the cells was determined by treatment with 1 2 N perchloric acid[17] and taken as 100% release

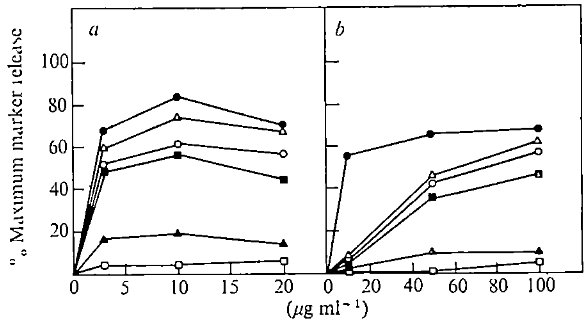

Fig 3 Polyene (*a*, amphotericin B, *b*, nystatin) susceptibility of liposomes derived from total lipid extracts of 139 (●), A1 (□), A2 (■), A3 (▲), A4 (○) and A7 (△) Liposomes with the test agents, as well as the blank control, were incubated at room temperature for 30 min The percentage maximum glucose released was calculated from the expression (glucose released in the presence of polyene — blank control)/(total amount of glucose trapped — blank control) × 100, as described previously[4 6]

We have found that effects on viability and cell leakage depend on temperature and phase of growth At room temperature (23°–24° C), nystatin and amphotericin B caused extensive killing and leakage of phosphate and ultraviolet-absorbing material from E139-A At 2° C, however, no cells were killed and we observed no leakage of phosphate and ultraviolet-absorbing material from them Similarly, cells in stationary

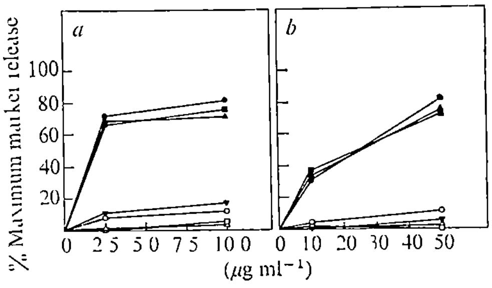

Fig 4 Polyene (*a*, amphotericin B, *b*, nystatin) susceptibility of mixed lipid liposomes Phospholipid and neutral lipid derived from one strain were mixed, respectively, with the neutral lipid and phospholipid derived either from the same strain (control liposome) or from a different strain (mixed lipid liposome) Experimental conditions were identical to those described for Fig 3 Neutral lipids of ●, E139-A, ■, E139-A, ▲, E139-A, ▼, A1, ○, A1, □, A3, △, A3 Phospholipids of ●, A1, ■, A3, ▲, E139-A control, ▼, E139-A, ○, A1 control, □, E139-A, △, A3 control

Table 1 Characterisation of polyene-resistant mutants of *C albicans*

		Polyene added (µg ml^{-1}) Strain	E139-A	A1	A2	A3	A4	A7
	Blank control	0 0	‡	†	‡	†	‡	†
	Amphotericin B	40 0	—	—	—	—	—	—
		20 0	—	*	—	*	—	—
		10 0	—	†	—	†	*	—
		2 5	—	‡	—	†	‡	—
Growth in liquid medium*		0 5	—	†	‡	†	‡	†
	Nystatin	100 0	—	†	—	†	—	—
		50 0	—	†	†	†	‡	†
		25 0	—	†	[illegible]	†	‡	†
		10 0	*	†	‡	†	‡	†
		2 5	‡	†	[illegible]	†	‡	†
	Nystatin	100 0	—	[illegible]	[illegible]	*	*	*
Growth on solid medium†	Nystatin*	100 0						
	Amphotericin B	20 0	—	*	—	*	*	—
Colour reaction‡			Greenish blue	Bright yellow	Purplish blue	Bright yellow	Greyish blue	Greyish blue
Doubling time (min)§			65	85	70	97	90	135

* Log phase cells were adjusted to approximately 5×10^4 colony forming units per ml in fresh growth medium, 2 ml of this dilution was added to tubes containing the given polyenes Dimethyl sulphoxide was present to 1% in all samples including the blank control After overnight incubation at 37° C, the turbidity was recorded

† A single colony of the given strain was streaked out on plate containing the given polyenes, and the growth was checked after 24 h of incubation at 37° C The nystatin (4530 U ml^{-1}) and the amphotericin B (876 µg ml^{-1}) were gifts from the Squibb Institute of Medical Research

‡ Liebermann–Burchard reaction used Total lipid extract of whole cells were used for assay Preparations from both log and stationary phase cells gave identical results

§ Growth was monitored turbidimetrically with a Klett–Summerson photoelectric colorimeter (green filter)

phase were affected far less than those in the logarithmic phase (Unless otherwise stated, only exponentially growing cells were used subsequently) These temperature and growth phase effects might be explained in three ways (1) changes of chemical composition in the membrane, (2) changes of effective pore size cell wall (a molecular sieving effect[16]), (3) a temperature-dependent metabolic process, operative only in growing cells, may be involved in the fungicidal action No decisive evidence favours any of these suggestions, and temperature dependence and growth phase dependence could be unrelated But, we have observed no appreciable difference in sterol patterns nor in the polyene-sensitivity of liposomes prepared from growing and stationary cells Furthermore, protamine (molecular weight approximately 18,500) can release ultraviolet-absorbing material from a strain of *C utilis* at both 30° C and 0° C (ref 17)

Table 2 Incorporation of sodium acetate-1-^{14}C into lipid fraction of wild type and mutant strains

	% Distribution of phospholipids PC	PE	PS+PI	Ratio of phospholipid/ neutral lipid*
E139-A	73 8	20 9	5 3	2 41
A1	60 6	28 0	11 4	1 74
A2	69 5	22 1	8 3	1 62
A3	68 7	19 9	11 3	1 63
A4	70 7	22 4	7 0	1 92
A7	76 5	13 1	10 4	1 46

Total lipid extract was subject to thin-layer chromatography on silica gel G plates with system $CHCl_3/CH_3COOH/H_2O/AcOH$ (65 43 3 1, v/v) Percentage distribution of phospholipids was calculated from the expression [(c p m of given phospholipid) (total c p m in phospholipid fraction)] × 100 PC, phosphatidylcholine, PE, phosphatidylethanolamine, PS, phosphatidylserine, PI, phosphatidylinositol

* Represent ratios of c p m in phospholipid fraction to c p m in neutral lipid fraction Preliminary experiments indicated that about 90% of the counts in neutral lipid fraction was associated with free sterols

Figure 1 shows the relationship of viability and leakage in mutants The release of cellular constituents did not result in or parallel extensive killing in mutants A4 (Fig 1*d*) and A7 (Fig 1*e*) The latter phenomenon is illustrated more clearly in Fig 2 In the presence of amphotericin B and nystatin, the viability of A4 was maintained, whereas E139-A was soon killed, the release of ultraviolet-absorbing material from both strains remained comparable If the alteration in permeability is a direct measure of membrane–polyene interaction, the data in Figs 1 and 2 suggest that there may be two mechanisms of polyene-resistance in yeast With type 1 mutants, A1 and A3, resistance arises from a reduced interaction between the cell membrane and the antibiotics as indicated by the low level of leakage With type 2 mutants, A2, A4 and A7, the antibiotics interact with the membranes, as evidenced by the altered permeability, but some critical step in the fungicidal effect fails to occur

The categorisation of mutants based on the loss of cell constituents can be reproduced in liposomal membrane systems derived from the total lipid extract of each strain (Fig 3) Liposomes prepared from mutants A2, A4 and A7 leaked glucose in a manner comparable with the wild type The apparent decrease in glucose release at high concentrations of amphotericin B was due to the interference of the antibiotic molecule at the absorption wavelength used in our assay Mixed liposomes were also prepared after fractionating total lipid extracts into phospholipid and neutral lipid components by silicic acid column chromatography[18] Figure 4 shows that polyene-induced permeability of E139-A was associated

Table 3 Mobility and percentage distribution of major sterol classes of wild type and mutant strains on thin-layer chromatography

	R_F values <0 14	0 32	0 48	0 55	0 67	0 72
E139-A	30 6	18 1		11 7	29 7	10 0
A1	38 8	8 8		18 2	22 4	11 8
A2	24 2	5 7	12 4	23 6	25 9	8 3
A3	45 2	7 4		15 5	21 2	10 8
A4	36 9	10 8		17 2	25 5	9 7
A7	24 4	13 3		22 3	27 4	12 7

Non-saponifiable sterol extracts were subject to thin-layer chromatography on $AgNO_3$-impregnated silica gel G plates The plates were developed half-way with benzene–ethyl acetate (2 1, v/v), dried under N_2 and redeveloped in the same direction the full distance with benzene–ethyl acetate (5 1, v/v) Figures given were calculated from the expression [(c p m of a given spot)/(total c p m in sterol fraction)] × 100

* As references, the R_f values of ergosterol and cholesterol are 0 32 and 0 55, respectively Sterols with polarities (as measured by their R_F values on silica gel) greater than ergosterol were not well resolved in this system

with the neutral lipid fraction (shown to be predominantly free sterol in preliminary experiments). Similarly the insensitivity of A1 and A3 to polyene-induced permeability changes was conferred on mixed liposomes by the neutral lipid fraction of these organisms. Our results clearly show that, in type 1 mutants, resistance arises from changed sterol patterns. Structural characterisation of major sterols in all strains is needed before this type of resistance can be discussed more specifically.

The existence of type 2 mutants indicates that leakage does not always result in killing. The discovery of nystatin-dependent mutants[19] suggests that this antibiotic could play a role in cell physiological function. The lethal action may involve polyene-induced malfunction of an essential physiological process or processes. When such a process is not operative, for example, at 2° C or when cells enter stationary phase or when a specific mutation occurs, its susceptibility to the fungicidal effect of polyenes may diminish. It has been reported[20] that iodoacetate and dinitrophenol reduce the fungicidal action of the polyenes on yeast.

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Crystallisation of a modified fibrinogen

MOST fibrous proteins do not crystallise. An artful way to promote protein crystallisation is to modify the structure by proteolytic attack. For example, papain cleavage of γ-globulin yields the homogeneous Fc fragment which readily crystallises[1]. Following a similar approach, we have crystallised fibrinogen.

Native fibrinogen precipitated at low ionic strength forms fibrous aggregates which are aperiodic in the electron microscope. After these precipitates had been stored in the cold for several months, we observed unusual rod-shaped structures which showed microcrystalline order in the electron microscope[2]. We had previously noticed that such preparations were often contaminated with bacteria, and it seemed likely that a bacterial protease had modified the native molecule so that it formed ordered aggregates. We cultured strains of *Pseudomonas aeruginosa* from such preparations and prepared a crude protease extract. This protease cleaved fibrinogen to a form that yielded highly ordered microcrystalline arrays[3]. We summarise here our studies on the effect of the protease digestion on fibrinogen and show that more extensive controlled cleavage leads to a modified molecule which forms crystals suitable for X-ray diffraction analysis.

The protease used in these studies was isolated from the growth medium of a strain of *Pseudomonas aeruginosa* grown on trypticase soy. The crude enzyme was salted out with ammonium sulphate. The protease was incubated at 31° C with native fibrinogen (5 mg ml^{-1} in 0.3M NaCl buffered to about *p*H 7) for various times. The reaction was terminated by addition of $10^{-3}-10^{-4}$M N-α-Tosyl-L-lysine chloromethyl ketone (TLCK), and the fibrinogen was dialysed to low ionic strength (5×10^{-3}M KSCN, 0.01M 2-(N-morpholino) ethane sulphonic acid (MES), *p*H 6.2). Samples were stored in the presence of the inhibitor and chloroform.

The progressive modification of the molecule was followed by measuring the percentage of mass lost as small trichloroacetic acid (TCA)-soluble peptides, and by observing the type and amount of ordered aggregates formed at low ionic strength. Four stages of digestion were distinguished. In the very early phase, corresponding to less than about 2% mass loss, most of the material precipitated out of solution at low ionic strength and consisted chiefly of aperiodic fibres, similar to native fibrinogen. With further digestion (corresponding to a loss of 4–6% of the mass as TCA-soluble material) the modified fibrinogen was more soluble, but within about 3 d as much as 85% of the material precipitated as microcrystals with a 450 Å axial repeat[3]. In the narrow digestion range between about 6 and 8% mass loss, precipitation was even slower, and both microcrystals and large birefringent crystals appeared; the proportion of crystals could be very high. On further digestion (about 10% mass loss) some amorphous precipitate formed, but most protein remained in solution.

The extent of protease digestion could be correlated with the appearance of the sodium dodecyl sulphate (SDS) gel electrophoresis patterns run in the presence of reducing agents[4]. The native molecule has a molecular weight of about 340,000 and consists of three pairs of chains designated Aα (~70,000 daltons), Bβ (~60,000 daltons) and γ (50,000 daltons)[5,6] (Fig. 1*c*). The Aα chain was attacked first. Microcrystalline samples, digested to about 4% mass loss, gave gel patterns with most of the Aα band and part of the Bβ band missing. The gel patterns of crystals showed a single heavily staining broad band with the same mobility as the γ chain of the native molecule (Fig. 1*c*). The clotability by thrombin of the molecules from the crystals was about 50% to 80% of that of native fibrinogen.

The crystals we obtained were thin birefringent plates with the longest dimension up to 1 mm (Fig. 1*a*). They were very fragile and characteristically cleaved in a direction perpendicular to the long axis to give rod-shaped fragments.

Preliminary X-ray diffraction patterns of single crystals taken at room temperature show reflections out to about 3 Å (Fig. 1*b*). The space group is $P2_1$. The unit cell dimensions are a=134 Å, b=97.3Å, c=174Å. β=92°20′. The density of the crystals is in the range 1.135–1.140 g cm^{-3} as determined by flotation of individual crystals in xylene–bromobenzene mixtures. Assuming that the partial specific

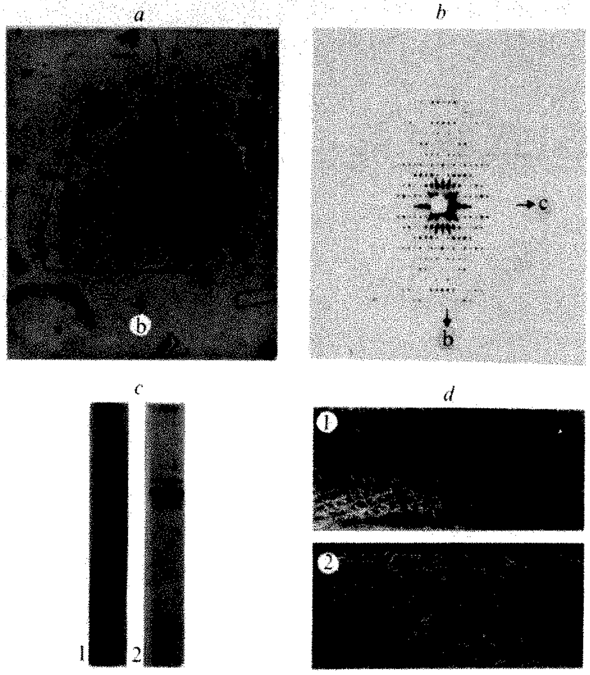

Fig. 1 *a*, Crystal of proteolytically modified fibrinogen. The b-axis is parallel to the long (vertical) axis of the crystal. Note small fragments cleaved perpendicular to this direction. (Approximately ×80.) *b*, A 4½° Precession photograph of of the Okl plane of the reciprocal lattice. The systematic absence of the OkO reflections with k odd indicates a twofold screw along the b-axis (vertical direction). *c*, SDS polyacrylamide gel electrophoresis patterns of (1) native fibrinogen; (2) modified fibrinogen from redissolved crystals. Both reduced with mercaptoethanol. *d*, Electron micrograph of crystal fragments showing (1) a net-like region: (2) fine diagonal, lateral and longitudinal striations spaced at about 45 Å, 60 Å and 35 Å, respectively. The sides of the mesh are about 105 Å by 115 Å; the acute angle is about 65°. Samples negatively contrasted with 1% uranyl acetate. (×190,000.)

volume of the protein is 0.72[7], the water content of the crystals is about 65%. The molecular weight of the protein in the asymmetric unit was therefore calculated to be 330,000, with an uncertainty of about 6%.

Crystals were washed, fragmented and negatively contrasted with 1% uranyl acetate for electron microscopy. Commonly observed images showed fine diagonal and longitudinal striations spaced at about 45 Å and 35 Å. More rarely, a net-like form was seen (Fig. 1*d*). The beaded strands of the mesh were about 30 Å in diameter, with the edges about 115 Å by 105 Å in length, defining an acute angle of about 65°. The negatively stained crystal fragments showed no direct correspondence with images of the microcrystalline forms which have a 450 Å axial repeat[3].

The unit cell dimensions and density of the crystals indicate a molecular weight for the modified protein of about 330,000 comparable with the native molecule. The estimate of mass lost from modified fibrinogen which crystallises is about 6–8% by measurement of the TCA-soluble peptides released during digestion. SDS gels of the crystals run in reducing conditions, where polypeptide chains separate by molecular weight, show material only in the γ chain region. Since no other large chain fragments are seen, the cleavage may split off relatively small fragments from the Aα and Bβ chains, reducing them to the size of the γ chain. The γ chain has a molecular weight of about 50,000; thus the weight inferred for the modified molecule from these gels is again about 300,000. Migration of the whole modified molecule on SDS gels without reducing agents present also gives a rough estimate of molecular weight close to 300,000. The chemical and crystallographic estimates of the mass of the modified molecule thus agree, within experimental uncertainties. This preliminary evidence on the modified molecule forming the crystal indicates that in spite of the cleavage it may be largely intact.

Redissolved crystals show a clotability about 50–80% of that of native fibrinogen. The fibres formed appear very similar to those of native fibrin in the electron microscope. This indicates that the modified molecule has retained the critical sites which interact to form the fibrin clot.

Some inferences about molecular shape and packing can be made at this stage, although the crystallographic data do not provide strong constraints. The modified fibrinogen crystallises in the monoclinic space group $P2_1$, and the unit cell contains two molecules of weight about 300,000. The molecule is a dimer of elongated shape, but a twofold axis is not a symmetry element of the crystal. From the symmetry of the crystal, these rod-shaped molecules must be arranged with two different orientations. Since the crystals readily cleave along the plane perpendicular to the b axis, the forces are weak between arrays of molecules related by the twofold screw. Only about 35% of the unit cell volume is occupied by protein, so that the molecules must form a fairly open meshwork of cross-connected rods.

The body diagonal of the unit cell in the crystal is about half the 450 Å length inferred for the fibrinogen molecule from studies of the microcrystals[3]. The dimensions of the unit cell in the crystal lattice need not, however, be related simply to the molecular dimensions. As in the assembly of other fibrous proteins[8–11] the rod-shaped molecules may pack with extensive side-to-side overlaps and end-to-end gaps, so that the observed periods may be only a fraction of the molecular lengths. The microcrystals of the modified fibrinogen molecule display a 450 Å axial period, and a specific staggering of arrays with this period can produce the 225 Å axial period and density distribution seen in the fibrin clot[3]. Although these large periodicities are not evident in the crystal lattice, it is likely that the modified fibrinogen in these crystals will show interactions similar to those in the microcrystals and in fibrin.

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Bringing men to the Moon

'TODAY scientific technology has reached the point where man has liberated nuclear energy and sent men to the Moon". Familiar, indeed trite, words that have been repeated a hundred times and which begin the preface to the handbook of the Third International Conference on the Unity of the Sciences to be held in London next month. But in this case there is an unintentional pun in it, for Moon is also the name of the man behind it all—Rev. Sun Myung Moon. And Mr Moon is an interesting character whose organisation seems to have a magic touch at compiling international gatherings stiff with Nobel Laureates.

The conference is sponsored by the International Cultural Foundation, founded in Japan in 1968 by Mr Moon, a South Korean. Its aims are "to inspire and foster the emerging world culture and civilisation", and to achieve this it lists prospective activities which would hardly be out of place at UNESCO—except that apparently the communist half of the world is not welcome.

The first conference was in New York in 1971 and twenty attended to hear Mr Edward Haskell propound his methods for eliminating "many defects produced as a result of the fragmentation of sciences as well as the breakup of science and morals . . . by viewing the scientific disciplines from a systematic viewpoint". "We had to listen to some extraordinary ideas on unification by way of Mendeleev's periodic table", said one participant. Another was horrified that whilst the scientists thought it silly, the philosophers present all took it very seriously.

Mr Haskell's influence seems to have waned between the first conference and the second, which was held in Tokyo last year with 60 participants discussing 'Modern Science and Moral Values'. Again there was international participation and for the second time there was plenty of money to fly the "deeply concerned scholars" to Japan. There were reply paid telegrams for those who didn't go, to send greetings for later reproduction as "messages of congratulation". Nobel Prize winners were carefully cultivated and identified as such whenever possible. And Mr Moon addressed the assembled gathering in very general terms.

Amongst the hand-outs to those who attended was a book *Unification Thought*. This book, written by an unnamed Korean, is published by the Unification Thought Institute of New York and is based on the Unification Principle of Mr Moon. In the second paragraph of the Preface we are told that "communism, which is a pseudo-value system, is eroding the Free World both in public and in secret". To counter this, Mr Moon's theistic ideology is described, with emphasis on Creation by God and the action of Divine Providence. The sweep of Mr Moon's thought is enormous and on the whole harmless. The Unification Principle "disregards the theory of evolution". The Unification Principle makes logical positivism "absurd", because of the "family Four Position Base". The "purpose" of the electron is to preserve atomic structure through revolving around the proton but also to bring joy to man indirectly. And so on. On values—the forthcoming conference is on absolute values—the writer concludes "there is no other way to find the true view of value than to perceive exactly what God's purpose for creation is". And the last chapter, on "Theory of History", begins to give us some idea what this purpose is.

History is, amongst other things, the history of re-creation and is marching towards a goal. Numerology then takes over.

First it is the number three. Everything reaches completion through three stages of growth. Three sons of Adam, three sons of Noah, three temptations of Christ and so on. Recently three stages of religious Renaissance, the first, Luther and Calvin; the second 17th and 18th century Pietism, Quakerism and Methodism; the third will develop soon on a world-wide scale when all religions and thoughts will be completely unified.

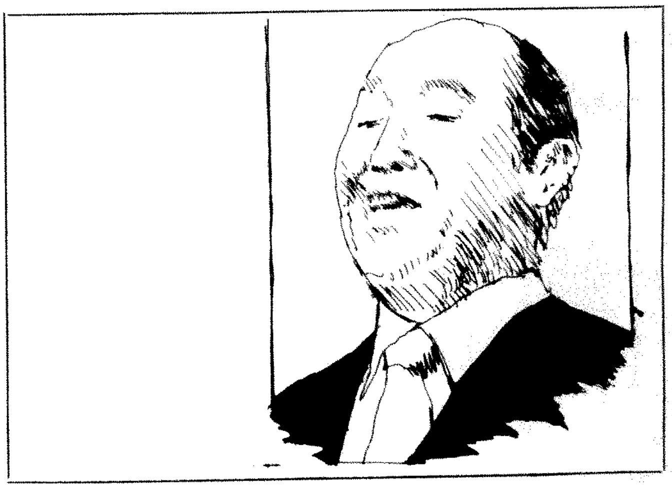
Then there is the number six. Six days of creation; six centuries of turmoil before Christ; six centuries before the appearance of the Third Adam. And the start of this last period? When the Pope became prisoner at Avignon, namely 1309. So the Third Adam should be around right now.

Next comes the number four in forty days, forty years, four hundred years.

Then, the law of false preceding true with Stalin as a false Messiah after whom we can "feel the approach of the Second Advent of the Messiah".

And finally the Law of Parallel Providence, comparing 1930 years of Old Testament development with 1930 years of development after Christ, again points clearly to an impending "Second Advent of the Messiah". So the Unification Principle exists to bring religion and science together in a unified culture and start a new cultural age under, presumably, a Third Adam or Second Messiah. Without the communists, of course.

Not that it is all theoretical. Mr Moon now has a following of a couple of million the world over and is at present crusading vigorously in the United States.



Large numbers of 'Moon children' have been imported from Japan and Korea and have affronted the American public by selling candles on street corners and showering New York and Washington with posters and pamphlets, thereby violating their visa status and breaking anti-litter laws. And large numbers of young Americans have taken it all seriously (*Time*, September 30). In the summer, Moon children were to be seen praying publicly in Washington for President Nixon. Just before a Madison Square Gardens appearance they ensured that Mr Moon had a large gathering of personal guests at a banquet at the Waldorf Astoria by camping down in United Nations missions to urge attendance on the diplomats.

The money for all this comes partly from street corner sales, but Mr Moon also owns several Korean businesses, making, amongst other things, ginseng tea and guns. The industries are given to him, it is said, by businessmen converted to the cause.

It is not clear that those invited to the Third Conference are aware of the background to the organisation. The initial brochure speaks of the purpose of the conference as discussion of the role of science in relation to problems of value and a possible way of setting up a universal standard of value for all mankind. It mentions Sun Myung Moon (without the Rev.) as the inspired founder. It lists British academic advisors, although not all on the list were very pleased to have their name used in this way. But it does not say that the fundamental philosophy is anti-communist, nor that the utterances of Mr Moon are such as to leave his followers with the impression that he himself is the Third Adam or Second Messiah.

The second round of publicity contains an extensive list of speakers—62 in all—and an even more extensive list of "commentators and discussants". No less than twenty-five of those attending have the words Nobel Laureate after their name. But none of the 150 listed comes from a communist country, nor even India, whereas three come from the Republic of China (Taiwan) and five from Korea (South Korea). A random check revealed that not everyone listed had ever had the intention of going to the conference.

The conference is being organised from an office in London. Mr Moon's book *Divine Principle* occupied pride of place on the mantelpiece. Questions were answered with charm and courtesy but generally in a rather hazy way; it could hardly be said that the philosophy of the organisation came through as anything other than the most vapid of generalisations about all sciences being one, science giving us the answers to help organise society, it not being desirable to use science for destruction and there being a spiritual dimension to science.

But have you tried to get representatives from the communist half of the world? Well it is not possible to invite people from all countries every year.

Is Mr Haskell, who played such a central role two years ago, coming this year? We aren't sure of that.

Who is this Mr Moon? (His name had modestly not been mentioned in our discussion before this point.) He is a Korean philosopher who inspired these conferences.

Is he coming? We don't know.

There is an empty slot on the packed programm for a Guest Address; who will give this? We shall b looking for someone soon.

For some, of course, the aims of this conference an its background are entirely in accord with their philosophy and they can hardly be criticised for giving it thei serious attention. And some who have been dissatisfie with the previous conferences have resolved to give i another try, in order to meet people, or in the hope tha the foundation has tightened up its academic credentials But many others seem to be in almost complete ignorance of the organisation behind it and unaware that thei presence may be used to confer some degree of respectability on something with which they would have littl sympathy.

Certainly the names of those expected to come shoul inspire some confidence that the operation will not b an entirely futile affair—provided they show up. Bu there are lingering doubts that everything is still not quit explicit, and there is an uncomfortable amount of usin of names and Nobel Prizes as a means of generatin momentum.

Never is an eminent scientist happier than when jetting around the world to conferences at which his eminence in one field permits him to lecture unchecked in another. One actually expressed the view that he would rather the conference had been elsewhere than in London to give him the chance for a trip. The foundation seems to have been successful in spotting two weak points of scientists—their Boeing complex and their propensity to amateur philosophising.

And a check with Mr Moon's Washington office reveals that he is indeed planning to be in London for the conference.

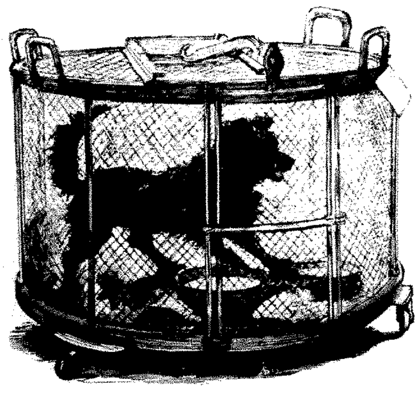
A hundred years ago

THE study of "seaweeds" is probably affected as much by the general public as that of fish; and whether or not the great mass of people who visit the Brighton Aquarium and other similar resorts really go there with any idea of becoming more intimately acquainted with the wonders of the deep, there is no doubt that the exhibition of varieties of ocean plants would be as popular as that of fish. A seaweed growing in water is very different from seaweed cast up on the shore, and a careful selection and arrangement of specimens would greatly enhance the interest of the tanks, while at the same time their presence would prove beneficial to the fish. We recommend the hint to the notice of the authorities of the Brighton, Crystal Palace, and Southport Aquariums.

From *Nature*, **10**, 530, October 29, 1874.

Mad dogs and Englishmen

The present epidemic of rabies in Europe is still spreading westwards and has now reached northern France; it could enter Britain. John Wilson has been looking at the risks to this country.



Caged and innoculated dog, 1884

THE most notable feature of the current outbreak of rabies in Europe is the involvement of wildlife. When the disease was eliminated from Britain and certain other European countries at the turn of the century, rabies had been maintained in the domestic dog. Now it is the fox population which acts as a reservoir of infection.

Whatever the reason, this new factor makes the present outbreak difficult to stem. As Britain's foxes are now more numerous than ever before, everything possible must be done to keep the disease away from them.

Rabies could reach Britain in two ways: a domestic animal incubating the disease might be landed illegally or an infected wild creature could make its way here under its own steam. The former is much more likely but although we can discount tales of rabid foxes attempting the channel swim, the second should not be ignored.

Bats can harbour rabies and certain species are known to arrive in Britain from the continent (the common long-eared bat, for example, migrates from Scandinavia to Yorkshire). Surprisingly, although rats are susceptible to rabies and carry other diseases from country to country on board ship, for some reason they have not yet brought rabies here. But as the distance between endemic areas of wildlife rabies and Britain diminishes, the likelihood of infection reaching us in these ways, however slight, must increase. And apart from greater vigilance against any animal stowaways, there is little that can be done to prevent it.

Illegal importation is another matter. According to a recent survey by the Ministry of Agriculture, Fisheries and Food (MAFF), 110 dogs, cats and other animals were landed illegally last year at the Kent ports alone. How many escaped detection is, of course, anyone's guess. Each of these animals might have been infected with rabies (some had been adopted only hours before the crossing) and transmitted the disease to pets and wildlife here. Although the report calls for greater deterrents against would-be smugglers, the greatest hope of keeping rabies out of Britain seems to lie in alerting the public to the dangers of the disease and in explaining why quarantine procedures are needed to prevent its entry.

And the public do need education; Britain has been free from rabies for several generations now and many people seem to think of it as a "disease of mad dogs". They have forgotten that rabies is almost always fatal in man once the symptoms appear—only two cures have ever been recorded—and so hideous are the agonies of the victim that euthanasia was once the common 'remedy'.

Nor are they aware of the regulations governing the importation of any animals they may acquire while on holiday. It is illegal to land an animal in Britain without an import licence and that is only given to owners who have made arrangements for six months' quarantine. Landing is held to have taken place before customs examination so even those people who declare their pet have committed an offence.

Official action in Kent last year prevented the entry of 33 animals but 10 evaded the port controls completely and were only detected inland (or on their way out of the country again). In all the other cases, it was the owners themselves who took action that prevented the animals from entering—and thus showed their ignorance of the law.

Customs officers reckoned that about 20 animals had been deliberately concealed. Perhaps the owners did so to avoid paying quarantine fee (about £120 for a dog) or perhaps from a misplaced sympathy of animals in quarantine. In either case, the fines handed out to the offenders (average £40) were hardly a deterrent. But under the Rabies Act (1974) fines have now been increased—£200 or more in some cases—and the local authority can have the animal summarily destroyed.

But if the worst comes to the worst how would Britain react to an outbreak? If pets or farm animals evaded quarantine there is some chance that the animal would be discovered before the disease had been transmitted to wildlife and to foxes in particular. That would be bad enough: family pets destroyed, valuable animals lost and people subjected to pain and anxiety. But if the disease did spread to the fox population things would be grave indeed. Continental workers have shown that rabies will still survive amongst foxes until their numbers are reduced to about 0.5 individuals per square kilometre. At this point the chances of a rabid fox infecting one of its fellows before it dies begin to peter out. Following the example of other European countries, the government would adopt a 'killer' policy, however unpopular it might be with some environmentalists.

With the exception of Denmark and possibly Belgium, this 'killer' policy has not met with much success. In France the disease is advancing along a front nearly 800 km long at the rate of roughly 30 km each year. To halt it would require a high degree of control over the fox numbers in a belt about 60 km deep—a prodigious effort. Britain is isolated, however, and the disease could be eliminated before it had taken a firm hold.

Plans are already prepared. Scientists from MAFF calculate that an area of about 12 miles radius around an outbreak of rabies would need to be virtually cleared of foxes. But before the hunting fraternity reach for their stirrups they should know that their activities would not be welcome for they could drive infected animals out of the area. An intensive poisoning campaign seems best.

Not everyone believes that the introduction of rabies to this country is inevitable but it is perhaps just as well that a new vaccine will soon be ready to treat any British victims of the disease. Developed at the Wistar Institute and produced at the Mérieux Institute in France the new killed virus vaccine is prepared from cultures of WI38 human tissue culture. Unlike vaccines now in use, such as the Duck Embryo Vaccine, it contains virus antigens and very little else—rather like the Salk polio vaccine. This should ensure that the incidence of side effects to vaccination will be greatly reduced.

Although trials of the WI38 vaccine are not yet complete the initial results are very promising. Even one injection quickly produces a very high titre of antibodies. The vaccine is expensive compared with conventional rabies vaccines but work is being done at the Clinical Research Centre, Harrow, to improve the dose regimen.

If the new vaccine passes its clinical trials only one painless vaccination in the arm would be necessary rather than the traditional 14 or so painful abdominal vaccinations now required. But no matter what improvements in treatment are on the way they are no excuse to take liberties with such a distressing and expensive disease. ☐

international news

Astronomers share Nobel Prize

This year's Nobel Prize for Physics was awarded to two astronomers, Professor Sir Martin Ryle (opposite) and Professor Antony Hewish (below).

Not before time, the Nobel Prize for Physics has been awarded for work in radioastronomy. Given that at last the prize was to go in such a direction, it could hardly have been awarded to anyone other than Sir Martin Ryle, who pioneered the technique of aperture synthesis (cited by the Nobel committee) which produced a revolution in radio astronomy techniques.

The long wait for Nobel recognition for any kind of astronomy (the award to Hannes Alfvén a couple of years ago was not strictly for his work as an astronomer) has encouraged various interesting rumours. Was it true, the more irreverent young astronomers wondered, that Alfred Nobel's wife had at one time had a liaison with an astronomer, as a result of which the inventor of dynamite and benefactor had excluded astronomers from the list of those eligible for an award? Alas for those who savour such gossip it now seems that those rumours can be dashed. But never mind, the significance of Ryle's work has itself caused enough confusion among the non-scientific press to enliven many an astronomical coffee break.

Perhaps the reaction of the *Wall Street Journal* best sums up the predicament of editors called upon to explain aperture synthesis for a lay readership: "Ryle refined the use of telescopes", it said on its front page, and left it at that. But the technique, like many great advances, is not so difficult to comprehend in retrospect—it is the ability to think of it in the first place which singles Ryle out for special mention.

The synthesis implied by the name of the technique involves using an array of relatively small radio telescopes to mimic some of the properties of a much larger instrument. The latest of Ryle's brainchildren is the 5-km instrument near Cambridge; there, a set of eight telescopes placed along a railway track is used to build up a picture of radio sources as they would be 'seen' by a single antenna 5 km in diameter.

This can only be achieved by making observations of the same source over hours, days and even longer. The 5-km 'aperture' is built up by the motion of the elemental antennas as the Earth rotates, and by judicious adjustment of their positions relative to one another along the railway track. You can synthesise most of the aperture for some of the time, and some of the aperture for most of the time; you can't synthesise all of the aperture for all of the time, but the result is that with a computer it is possible to provide a map of the average intensity of the source as viewed by a non-existent 5-km dish.

So it is no surprise that Ryle's work has been recognised in this way. He already has just about every honour available to a British scientist, having been elected a Fellow of the Royal Society in 1952, appointed to the first chair of radioastronomy in the University of Cambridge in 1959 and knighted in 1966; he became Astronomer Royal in 1972. Not least among his many achievements are his teaching skills, which have probably contributed as much as his scientific ability to making the Cambridge radioastronomy group one of the best in the world.

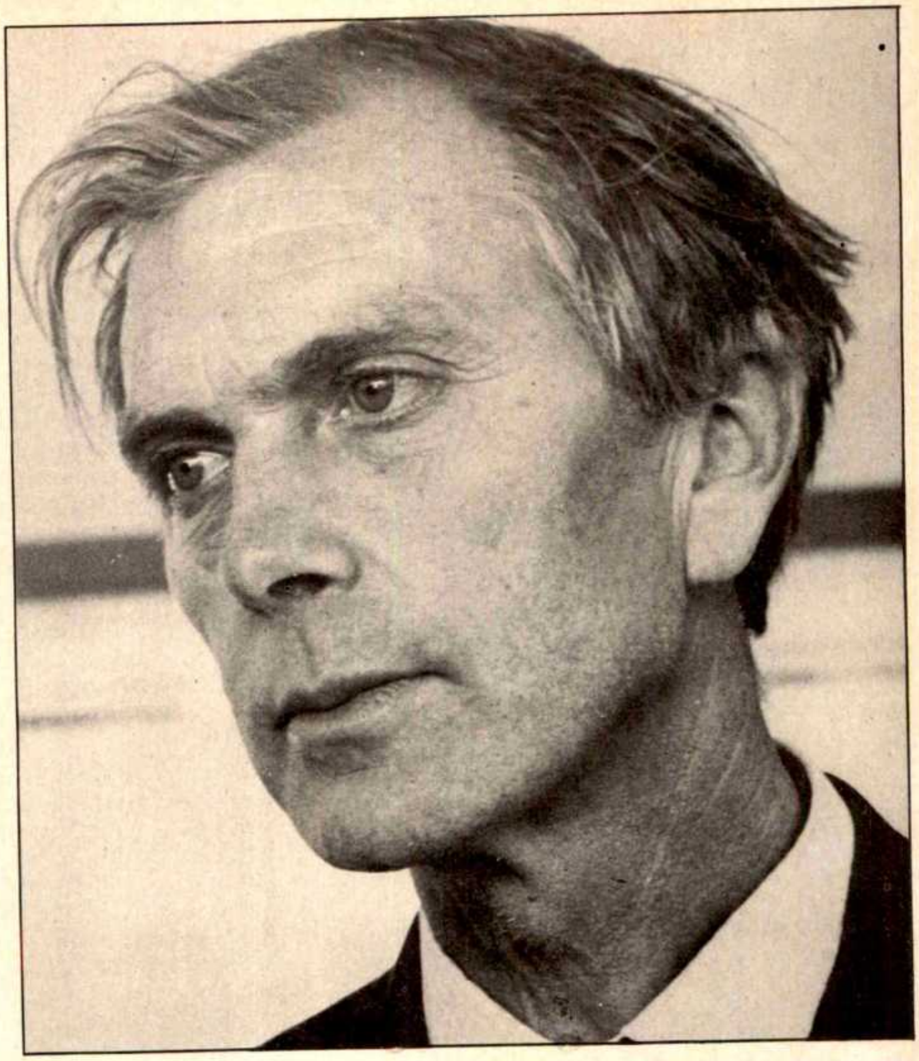
John Gribbin

The communication from Hewish and his students which reached the offices of *Nature* on February 9, 1968 will surely stand as one of the classic contributions to science in this century. Even to those of us accustomed to a succession of remarkable discoveries by radioastronomers, the news that there were objects in the Universe emitting radio waves in pulses, regularly spaced with intervals of about a second, strained credulity. A few days before this paper appeared in *Nature*, Fred Hoyle told me during a meeting in London that Hewish had discovered this new kind of radio source. My instant reaction was one of disbelief, an instinctive feeling that nothing in the Universe could behave like this and that his system must have been confused by the all too familiar problem of terrestrial interference. On the contrary the discovery was absolute and, in a sense which is rare in science today, the unique property of Hewish and his colleagues.

Why did Hewish find these signals when the rest of us with far more expensive and complicated radio telescopes missed them completely until we knew where and how to search, when they became obvious immediately? Hewish was studying the phenomenon of interplanetary scintillation. The effect, which he had discovered in 1964, occurs when the radio waves from a small diameter source such as a distant quasar are diffracted as they traverse the plasma clouds in interplanetary space. This induces a fluctuation in intensity with a period typically of about a second on otherwise steady signals. The study of this phenomenon was proving to be extremely useful both for the investigation of the solar corona and also for the measurement of the angular diameter of the distant radio sources. One of the last acts of the Department of Industrial and Scientific Research before it was disbanded in 1965 was to award Hewish a grant of about £17,000 to build a special aerial array which he had designed specifically for these investigations. There were at least three critical features of this concept which predestined that Hewish would discover objects in the Universe for which he was not searching. The aerial was very large, consisting of over 2,000 dipoles it covered 4.5 acres, and therefore had a far greater collecting area than any of the synthesis aerials at Cambridge, or of the steerable radio telescopes elsewhere. The receiver and pen recording system was designed with time constants to record fluctuating signals with periods of the order of a second. Finally, whereas radio astronomers all over the world were straining to work at shorter wavelengths in order to achieve more esolution with reasonably sized aerials, Hewish remained interested in the metre waveband where the plasma effects are more pronounced than at horter wavelengths.

In the event, the DSIR grant turned out to be one of the most cost-effective n scientific history. Less than two months after recording began, Hewish's student Miss Jocelyn Bell noted the recurrent pulses on the recording charts around local midnight when the normal scintillation phenomenon is inconspicuous. The drama of the succeeding months as Hewish established the extraterrestrial and stellar nature of the signals was hidden from the world until there could be nothing but astonishment at the final outcome. Six years later with 120 pulsars under study there seems little doubt that the signals have their origin in neutron stars, the end of a supernova explosion—a possibility suggested by Hewish in the initial publication. In these events the remains of the star have collapsed into a body with a diameter of only tens of kilometres, with densities of the order 10^{14} g cm^{-3} and possibly with magnetic fields of 10^{12} gauss.

It is this state of matter, hitherto merely a speculation of theorists, which is now observable, and the discovery must surely be acclaimed as entirely appropriate to the distinction now accorded to Hewish. BERNARD LOVELL



Royal inauguration for AAT

from Peter Pockley

As if to show that astronomical research is truly an integral part of Western civilisation, the largest optical telescope in the Southern Hemisphere was officially opened on October 16 to the strains of a string quartet playing Purcell, Dvorak and Mozart. Prince Charles came from England to say the words which set the massive 326-tonne telescope slewing and the 526-tonne dome rotating in a bravura display of engineering gymnastics which left the 400 official guests (comfortably seated on the floor of the telescope) applauding in amazement at the scale of it all.

The ceremony was nicely done. The engineers and scientists were at pains to show that they can turn on a show worthy of princes and premiers and the $A16 million of public money which it cost to build. For the people of the modest country town of Coonabarabran (population some 3,000), 29 km away, there had never been such a day. The town's five taxis had a field day ($A3 to the airport, $A8 to the telescope), the flags were out, the schoolchildren and the farmers met a real Prince, and the security men, obvious in their plainclothes, were there in force, giving rise to endless gossip.

Charter and VIP flights brought hundreds from Sydney (340 km to the south-east) and Canberra. There was a batch of 16 British scientists, too, led by Professor Sir Fred Hoyle, present Chairman of the Anglo-Australian Telescope (AAT) Board, and Professor Sam Edwards, Chairman of the Science Research Council. They will have to work hard to keep up an Anglo semi-image to the project—but there will be no trouble over control and access to the telescope which was established on a very formal intergovernmental level with 50% shares each. In the popular imagination the AAT will always be depicted in the midst of the gum tree landscape of the Warrumbungle ranges, spectacularly spread out beneath Siding Spring Mountain which is the 1,165-m-high platform for the big dome and its four smaller companions (three Australian National University reflectors and the United Kingdom's own Schmidt telescope). Despite the careful 50/50 protocol of the opening ceremony and the words of Prince Charles and Prime Minister Whitlam, the AAT will certainly become an Australian national symbol.

The description "largest in the Southern Hemisphere" is relatively easy to come by with feats of technology, for the land mass and population south of the Equator are but tiny fractions of the world totals. Yet this telescope is genuinely in the big league, and the situation of this giant eye where it can look right into the centre of the Galaxy and the Magellanic Clouds, the nearest galaxies to us outside our own, will give it an edge on its competitors (and collaborators) in the north which is sure to produce a spate of important observations. In the competitive astronomical stakes, though, the American 3.9-m telescope at Cerro Tololo in Chile will be only a few months behind the AAT.

The first calibrating photographs have already been taken before the mirror gets its first aluminising by the end of this month. Professor Ben Gascoigne, the Commissioning Astronomer, has enthusiastically pronounced the optics to be first class, better even than the specifications. He has now been joined at the AAT by the first Director, Joe Wampler, from Lick Observatory.

One European required for Spacelab

from Angela Croome

AT least one European scientist must be chosen in the next six months to accompany and tend the European experiments aboard the first Spacelab—the orbital workshop designed and built in Europe, which is to be raised into space and returned by the American Space Shuttle, the prime space effort in the United States for the 1980s.

This is a deliberate phase-shift of a size that is scarcely appreciated, so quiet has been the final transition from automated satellites to a reusable man-in-space project. Europe seems to be slipping into the manned space programme almost by the backdoor. Yet a series of papers of the recent Astronautics Congress in Amesterdam show how fast the options are closing and what a brisk pace is being set.

The subtle combination of reliability, systems compatibility, miniaturisation, ruggedness and strict programme planning and management that space operations demand are recognised as an industrial challenge compatible only with a major weapons development or a nuclear programme and a means of forcing the pace both of technology and of management. This indeed has been one of Europe's principal motives for getting into the space business in a substantial way. Added to these are the implications of man-in-space, in an international context specifically. The requirements of a manned spacecraft are at least an order of magnitude more severe than for automatic satellites. Pressurisation, life-support, toxicity and flammability restrictions must all be considered, inevitably increasing design complexity.

The first Spacelab will fly in the spring of 1980 and the final choice for this mission must be made in the spring of 1975. In fact the experiments and the man-in-space to run them are a composite package. If, for instance, a major University of Southampton experiment in atmospheric physics requiring constant supervision is selected, then a Southampton experimenter would be expected to accompany the instrument and operate it. This means, say the NASA space medicine experts who have overall responsibility for man-in-space survival and performance, that Spacelab scientists will have to be part-qualified as astronauts to complete their tasks under zero-*g* and other disagreeable and unfamiliar space conditions—in short they will have to be under 55 and obtain experience of piloting high performance jet aircraft. An interesting challenge for typical European departmental professors, one can say. The intention is that the first Spacelab payload will be shared roughly equally between Europe and the United States, with NASA having the final say in what flies and what does not.

More than 200 European proposals for the first Spacelab payload have already been received. In July the European space agency, ESRO, issued 200 invitations to appropriate industrial and academic bodies in Europe to put up ideas. Responses were due by the end of August but they are still flowing in. The German company Messerschmitt-Bölkow-Blohm is carrying out a preliminary screening, which includes tossing out proposals that are hopelessly incompatible or take insufficient advantage of Spacelab's special opportunities for bulky and relatively power-hungry experiments, and it will make its recommendations to ESRO in December. ESRO and NASA will then get together to define the first mission precisely and the final choice of experiments will follow from this.

The breakdown of the initial European proposals is interesting. By far the most numerous are technological and industrial rather than strictly scientific. (One German company has put up 20 proposals alone relating to communications.) The largest category is for space processing; 80 ideas have come in so far (more than twice as many as in any other category). There has been great interest in last year's American Skylab experiments in which pure crystals of such elements as germanium used in electronics components grew rapidly to diameters as great as 30 cm—impossible on Earth where growth is inhibited by gravity. There is speculation that space-grown crystals could actually bring down the high price of electronic components such as transistors and panels for solar heating. The production of special quality welds and of exotic alloys is also a possibility that European technologists wish to explore. Chemical separation by electrophoresis, possibly only under weightless conditions, promises greater purity in drugs and vaccines. Even an experimental space factory (requiring as it does bulky equipment, considerable power and a man in charge) has been outside the range of Europeans previously.

Earth resources and earth surveying is the second largest category of applications. It includes a novel experiment using a laser and a telescope to measure atmospheric scattering of light by gas particles; it will weigh two tons and consume one kilowat of power. Two-thirds of the Earth-monitoring proposals come from Britain. Skylab experience has shown that a man in charge is a great asset for this type of survey from space for he can react fast—for instance, refocus his instruments for detailed observation—when a special feature crops up.

The life sciences stand to benefit particularly from a supervised orbiting laboratory and there are plenty of ideas. A biological experiment is typically small and weighs little but it is expensive in human time.

The first Spacelab flight will be atypical. It will not orbit for more than a week and half the available power will be used to check out the subsystems and procedures. It will be primarily a technical flight to make sure everything works. Consequently two out of the four payload staff will be equipment technicians, presumably provided from the American side. But the timetable for the second Spacelab lags only three months behind the first and 40% of all shuttle flights are expected to carry a Spacelab. Europe is now inextricably involved in the major manned programme for the 1980s and is being paced on a sharp timetable by NASA. For the United States, the space programme has proved a forcing house in engineering and management. Manned missions raise the game more for they cannot be allowed to fail. □

Ariel-5

THE fifth in a succession of British scientific satellites which have been put up by NASA, in the absence of any suitable indigenous rocketry, was successfully launched this week from the Italian San Marco platform off the Kenyan coast. Called Ariel-5 (UK-5 before the launch) the satellite is only the second in the world to be given over entirely to X-ray astronomy, although individual X-ray experiments have been carried on other satellites.

Ariel-5 has six experiments on board, five British and one American, and information will be fed to and from the satellite by the Science Research Council's Appleton Laboratory at Slough. Four of the experiments, designed by University College, London (two), University of Leicester and Imperial College, London, will be pointed at specific X-ray sources whereas the other two (University of Leicester and Goddard Space Flight Center) will scan large areas of the sky and suggest where it might be particularly interesting for the pointing experiments to look. X-ray sources which turn on unexpectedly will be monitored in this way.

Mr J. F. Smith, Programme Manager for Ariel-5 in Britain, said this week that the satellite is functioning extremely well. All the systems are working, he said, and the only problems are minor ones which will be ironed out in a few days. One group already has interesting scientific results to hand. □

correspondence

Checks and balances

SIR,—You raise the issue (*Nature*, September 6) of the role of journals in situations such as the proposed moratorium on plasmid engineering experiments You conclude for two reasons that you should not, as a policy, refuse to publish any report of experiments that might be carried out in defiance of the call for postponement—this call, or others like it Your two reasons were scientific variants of classic arguments against censorship what referee can judge papers for their ethical standing? Why drive publication underground? Yet you acknowledge implicitly the considerable power of journals such as yours to influence the standards—scientific, but perhaps ethical as well—of researchers Is there a way to use your power not through editorial censorship but instead as an important auxiliary to the checks and balances that exist or may need to be set up in the scientific community? For a model, consider the statement you will find in the instructions to contributors to *Proceedings of the National Academy of Sciences* "Manuscripts reporting experiments with humans should be accompanied by a copy of the document authorising the research by the responsible institutional committee "

American research hospitals and medical laboratories, as is generally known, have peer committees that must pass every plan for an experiment using human subjects, while there is still time—that is, before the experiment is begun But one important aspect of the open letter by the American group was that the danger was imminent, as Paul Berg, a leader of the group, has said several times, many of the experiments at issue were already planned to be completed before the international meeting in February, and their dangers if any, reside not in some future consequence or application of published results but in the experiments themselves Control must occur before the work, not merely before publication—not *imprimatur* but *laboretur* The nuclear fission or "Hahn should not have published" analogy is misplaced But many American and British biological laboratories, whose own people would be the first victims of an experiment gone wrong, are setting up biohazard or biological safety committees—scientific peer groups who will review research plans Such review is decentralised, appropriately motivated, and can be done early enough, it is done not just by peers every couple of years (as with peer review of grant applications in the American setting) but from week to week by colleagues who ought to be familiar with men, their work, and their equipment Such institutional structures seem to me to be admirably adaptable to part at least of the present problem As local safety committees begin to function, to reinforce them with an editorial requirement that reports submitted for publication be accompanied by evidence that the local review was done—like the rule at *PNAS* for human subjects—that would be a natural step with no taint of censorship

Yours faithfully,
HORACE JUDSON

Manting House,
Meldreth House, Cambridgeshire

Joke with whiskers

SIR,—Martin C Raff (Nature, 251, 184) describes nude mice which have spread through the immunological world at a remarkable pace Should they be called squeakers?

Yours faithfully,
M A PERKIN

Rochdale, UK

For the birds

SIR,—Dr W R P Bourne in his article "Egg Collector Collected" (*Nature*, **249**, 793, 1974) has done well to draw the scientific world's attention to the egg- and bird-collecting outrages regularly perpetrated by ornithologists, some of high repute, in North America I can vouch for his reference to a "conspiracy of silence", leading publications in the field have declined to publish any mention of this disturbing subject It is perhaps time that the rest of the world was made aware of the North American situation

One might imagine that the National Audubon Society (NAS) would take a stand against this After all, in the early days, were not two Audubon wardens murdered in defending egret nests in Florida? Yet when in 1969 two biologists shot 1,000 egrets in that state in the space of four weeks[1] this action was defended in a letter to me from the NAS by saying that it was all right because it was done by the "proper authorities"

What has been overlooked, however, is that experts in population dynamics (if experts they are) are not the only people with an interest in birds To a great majority of people there are ethical and aesthetic considerations involved in taking the lives of so many beautiful and harmless creatures This is perhaps an unscientific attitude, but birds do not belong to scientists and many people see no reason why, as in North America, ornithologists have some prescriptive right to kill them

One might imagine that no ornithologist would raise any serious objection to a code that asked a collector to state, when applying for a permit, the nature of the scientific research he was engaged upon, what new information and understanding about avian biology he expected to gain from a study of his specimens, what species he required and how many, whether such information could be gained from a study of pre-existing specimens, and whether his collecting was likely to harm any population The permit-issuing agency might then assess the scientific merit of the proposal and then issue a permit for a specified number of a named species for an approved purpose Yet this suggestion has been repeatedly rejected by ornithologists in North America Applicants are not obliged to state which species they wish to collect, open permits are issued without specifying which species may be shot or how many, and their holders may shoot any birds regardless of whether they are engaged in research on them or not

Yours faithfully,
J B TATUM

Department of Physics,
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Columbia,
Canada

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[1] Fogarty, M J, and Hetrick, W M, *Auk*, **90**, 268 (1973)

Blameworthy

SIR,—In my article last week ('For those in peril on the factory floor') the Royal Society was unfortunately omitted from the list of organisations taking no interest in asbestosis and similar consequences of technology In fact the Royal Society should have headed the list as the body with the greatest influence but the least inclination to do anything about it

PETER J. SMITH

news and views

In pursuit of the transforming genes of adenoviruses

ANALYSING the functional activity of any large pieces of DNA seemed a prohibitive task until the advent of bacterial restriction enzymes They have, without question, revolutionised the field of tumour virology, which was moving fast enough before their arrival, but which now produces important new facts at the drop of an enzyme This is because, although the overall function of some animal tumour viruses, particularly of the papova and adenoviruses, was quite well understood, mapping of gene function by conventional genetics was proceeding very slowly, through surely A method of selecting specific pieces of viral genomes to use as molecular probes has provided a way round this difficulty With such probes it is immediately possible to say, for instance, whether the fact that transformed cells do not show complete virus expression is because the complete viral genome is not there And it some of it is lost, then presumably that which remains contains those genes responsible for inducing or maintaining (or perhaps both) the transformed state Questions such as these are at the core of current efforts to demystify the process of transformation, which, many people hope, itself bears some resemblance to tumour induction *in vivo*

First to yield to mapping by restriction enzyme cleavage was the genome of SV40 Lagging somewhat behind, but now catching up rapidly, was analysis of the larger adenovirus genome Three new papers highlight recent developments in the adenovirus field, using specific pieces of the genome produced by restriction enzymes of *Escherichia coli* (*Eco*RI) and of *Haemophilus parainfluenzae* (*Hpa*I) Gallimore, Sharp and Sambrook (*J molec Biol*, **89**, 49, 1974) have analysed the viral DNA in lines of rat cells transformed by adenovirus 2 (Ad2) Their work, together with that described in a previous paper (Sharp, Pettersson and Sambrook, *J molec Biol*, **86**, 709, 1974), has led them to the conclusion that no Ad2-transformed rat cell contains the whole viral genome This conclusion could not have been reached without specific pieces of adenovirus DNA to use in reassociation experiments, which are conducted as follows Labelled probe DNA (whole or fragmented Ad2 DNA) is reannealed in the presence of unlabelled transformed cell DNA, and the increase in rate of reassociation produced by the viral sequences in the transformed cell DNA is measured This increase is proportional to the number of viral genomes present But using whole viral DNA as probe, there is no way of distinguishing between about one whole copy of the viral genome per cell and many copies of a small part of it—or any of the intermediate possibilities Also, the frequency of different sections of the genome may vary, giving increases in reassociation rate which are averages If restriction fragments are used as labelled probes instead, then the problem is vastly easier to disentangle *Eco*RI enzyme produces six fragments of Ad2 RNA, one of which (fragment A) represents 58 5% of the genome, and is at the left-hand or GC-rich end *Hpa*I enzyme produces seven fragments, four of which (fragments E,C,F and A) are in the *Eco*RI A fragment section of the genome This is useful since a combination of *Eco*RI and *Hpa*I treatment of adenovirus DNA allows dissection of *Eco*RI A fragment function With all these thirteen fragments as labelled probes, Gallimore *et al* measured their reassociation rates in the presence of DNA from nine different lines of transformed rat cells Six lines show essentialy the same situation they contain sequences complementary to about 14% of the viral genome, all of which are at the left end and represent all or part of *Eco*RI fragment A, and *Hpa*I fragments E and C The number of copies of these sequences apparently varies between lines though, as the authors point out, this may be due to variations in the ploidy of the cells, which would make the figure used for DNA content per diploid cell inaccurate Three other lines of transformed cells examined at the same time contain larger proportions of the adenovirus genome No line contains a complete genome, and no line lacks the sequences at the left-hand end

These findings are significant for a number of reasons First, for lack of evidence to the contrary, people have tended to assume until now that transformed cells contain at least one viral genome The only suggestion that this might not be so has come from the fact that there are transformed cell lines from which virus cannot be rescued by any of the available techniques All the lines used by Gallimore *et al* fall into this category It is interesting that rat cells are also semi-permissive for adenovirus Gallimore *et al* argue that those cells which survive and become transformed are those which, for one reason or other, contain defective viral genomes Possible reasons are either that the original infectious particle was defective, or else that part of the genome was lost after infection

If the presence of viral genes is necessary for the maintainance of the transformed state, then the work of Gallimore *et al* means that only 14% of the adenovirus genome is involved, since several of their cell lines contain only this much of it Another way of approaching this problem would be to use specific fragments of the genome to induce transformation It may be that the same left hand 14% of adenovirus DNA which is present in transformed rat cells contains all the information necessary to induce transformation in the first place A year ago, Graham and van der Eb (*Virology*, **52**, 456, 1973) described a technique for assaying infectivity of Ad5 DNA and showed that it could transform rat cells (*Virology*, **54**, 536, 1973) In this issue of *Nature* (page 687) they report some results of their efforts to localise the transforming genes by using sheared adenovirus DNA as the transforming agent The technique involves forming a co-precipitate of the DNA and calcium phosphate, which settles on to the cells, and from which DNA is taken up Graham *et al* demonstrate that whereas infectivity of adenovirus DNA is very sensitive to shearing, transformation can be induced by pieces only 1 0 $\times 10^6$ daltons in size—about 5% of the whole genome (20–25 $\times 10^6$ daltons) Separation of the two halves of the genome (which have an asymmetrical distribution of GC) reveals that it is the left or GC-rich half which has most of the transforming activity In attempting to localise this further, Graham *et al* have followed the effect of progressive enzyme digestion from the two ends of the genome inwards They conclude that because transforming activity is not affected

A sketch of Tasmanian Aboriginals done from memory by Annie Benbow in 1900 The last member of the race died in 1876, they had been moved from Tasmania to Flinders Island in the Bass Strait and the last 44 returned to a former convict probation station in Tasmania in 1847 Most of them died of influenza (Records of the Queen Victoria Museum No 49, 1974)

by digestion of the terminal 1% of the left end, but then decreases rapidly on further digestion, a segment extending from 1% in to about 6% of the genome comprises the transforming or 'T' segment The results do not, however, really justify this degree of precision in defining the T segment Its function could, for instance, be affected by destruction of sequences adjacent to it, and its actual position may not be so close to the left end as it seems A much more convincing approach would be to use restriction fragments of adenovirus RNA as transforming agents For instance, the *Hpa*I E fragment makes up 4 1% of the left end of the genome and it, together with some of the adjacent fragment C, should contain the T segment In the discussion at the end of the paper, such studies with restriction fragments are said to have been done, I hope their outcome will soon be published

The work of Graham *et al* reinforces the conclusions of Gallimore *et al* that it is the left end, and probably the left 14%, of the adenovirus genome which is concerned with cell transformation The experiments of Graham *et al* provide a way of studying the induction of transformation by pieces of DNA which cannot code for more than two proteins, and may only code for one The transcription of these sections of adenovirus DNA in transformed cells is now of even greater interest Previous work has already shown that only a small percentage of the viral genome is transcribed in some adenovirus-transformed cells—a phenomenon also well documented for other DNA tumour viruses The possibility that this is because most of the genome is actually missing as opposed to being repressed is an exciting one

Another technique which has been essential to recent experiments on SV40 transcription is the separation of the two complementary strands of SV40 DNA (Westphal, *J molec Biol*, **50**, 407, 1970) The technique depends on the asymmetrical transcription of twisted circular SV40 DNA by *E coli* RNA polymerase Using separated strands, it has been shown that sections of both strands of the DNA are transcribed during the lytic cycle (Khoury, Martin, Lee, Danna and Nathans, *J molec Biol*, **78**, 377, 1973) It is not possible to separate the two strands of adenovirus DNA in the same way Whole strands can be separated by differential binding of poly(U,G) to denatured Ad2 DNA followed by density gradient centrifugation Tibbetts and Pettersson (*J molec Biol*, **88**, 767 1974) have now published a combination of this technique plus some kinetic trickery which produces labelled single strands of all the six *Eco*RI restriction fragments of adenovirus DNA The technique is as follows Large amounts of unlabelled whole complementary strands of Ad2 DNA are prepared as described above A large excess of one whole complementary strand, for instance the heavy strand, is then mixed with labelled Ad2 DNA (whole or a specific restriction fragment), denatured and exhaustively renatured What happens is that in the presence of a large excess of unlabelled heavy strands the amount of labelled complementary light strands becomes limiting, and at the end of the reaction up to 50% of the DNA remains single stranded This residual single stranded DNA can be isolated by hydroxyapatite chromatography and corresponds to the heavy strands present in excess during the reaction The important point is that it includes both labelled and unlabelled strands, because the unlabelled heavy strands, being present in large excess, will preferentially renature, so effectively excluding their labelled counterparts from re-forming double-stranded molecules with their original partners, the labelled light strands The technique of Tibbetts and Pettersson produces very pure single-stranded preparations, and much of their paper is concerned with establishing this and with analysing the kinetics involved They then go on to use their preparations to show that in HeLa cells productively infected with Ad2, early cytoplasmic mRNA is complementary to 15% of the heavy strand and 30% of the light strand Late mRNA is complementary to 22% and 66% respectively Of this late mRNA, most is transcribed from both strands of *Eco*RI fragments A,B and C, and from the light strand of fragments D,E and F They discuss the implication that these areas of the genome may represent groups of genetic functions Now the use of single-stranded restriction fragments should be extended to analyse transcription in adenovirus-transformed cells

The three papers discussed in this review illustrate the application of three important techniques, namely, the use of restriction enzymes to produce specific fragments of viral genomes, the use of fragmented viral DNA to induce cell transformation, and the separation of complementary strands of viral DNA to study viral transcription They also herald what could be the final campaign in the battle to analyse transformation from the viral point of view Unfortunately, of course, transformation involves cells as well Whether, when the viral DNA that is present in transformed cells is fully understood structurally and functionally, we shall also understand how transformation is induced and maintained remains to be seen Tumour viruses should perhaps be considered as particularly useful triggers—because they can be analysed—for inducing cells to lose control of their division and growth What we shall have when we understand their function is a model for studying essential cellular control mechanisms The implications of this are immense

Elizabeth Rogers

Fighting foot-and-mouth

from a Correspondent

THE Animal Virus Research Institute celebrated the completion of 50 years of research on foot-and-mouth disease at Pirbright by holding a symposium on September 26 and 27

The changes in the world situation were reviewed by three speakers Dr J G van Bekkum (State Veterinary Institute, Lelystad, Netherlands) presented the tables of prevalence of the disease in European countries in the last 50 years During the major waves of disease—for example, in 1937/8 and in 1951/2—there were as many as 370,000 outbreaks in countries such as France and Germany The development of the tongue tissue vaccine by Frenkel had made possible the introduction of prophylactic vaccination and campaigns were begun in Holland in 1952 and, within the next ten years, in France and Belgium In each country the number of outbreaks fell dramatically, to under 10 yr^{-1} There had been problems in relation to immunisation of pigs but these were being overcome and, in the recent outbreak in France, vaccine had played an important part in control Europe still remained at risk through importation and through possible spread of the disease from Eastern Mediterranean countries but prospects for the control of ultimate eradication from Western European countries were good

Dr J H Graves (Plum Island Animal Disease Research Laboratory, New York) dealt with current problems in the control of the disease in the Americas In spite of the great efforts which have been made in extensive vaccination campaigns, results have not been as good as would have been expected on the basis of the European experience A particularly difficult problem was the control of potency of vaccines used in the field and it was here that the work of the Pan American Foot-and-Mouth Disease Centre in Rio de Janeiro might be expected to influence the situation This centre had already run many courses for laboratory workers in various countries in South America and, as a result, diagnostic methods had been improved and standardised Similar action is now being taken on vaccine potency control Dr Graves also referred to the new risks which were posed by the planned Pan-American Highway Plans were being discussed for quarantine zones to prevent spread of the disease northwards

Dr J B Brooksby (Pirbright) drew attention to the very uneven progress in control of the disease in countries in Asia and Africa Extensive campaigns have been carried out in the Soviet Union but the statistical information available is slight In many other regions limited campaigns have begun Perhaps the most encouraging of these were in relation to disease-free zones A good example was in Kenya, where a start had been made with a limited number (fewer than 400,000) of animals and gradually extended as the campaign was shown to be a success The zone in which the disease was not controlled included nearly two million animals Though the total number of animals so far involved in Asia and Africa was trivial in comparison to the population, it was in such schemes that lay the greatest hope for ultimate control of the disease and the development of useful programmes for livestock improvement

In another session, the biosynthesis of the virus and the structure of the virus particle in relation to its antigenic properties were reviewed by members of the biochemistry department of the institute A paper by Dr K M Cowan (Plum Island Animal Disease Research Laboratory, New York) carried the studies of virus properties into the field of vaccines The properties of two plaque variants isolated from cultures of virus of type Asia 1 in BHK cells were described Selection occurred during routine passage, the large plaque variant being predominant in monolayer culture, where as the small plaque variant was favoured by growth in suspended cells The small plaque variant was antigenically deficient in serological tests and also in its capacity to immunise cattle This finding emphasised the practical importance of the method of culture of the virus for vaccine production

The arithmetic of destruction

by David Davies

RARELY does the arithmetic of strategic nuclear weaponry surface for public discussion words such as superiority, sufficiency and imbalance are reckoned to be all that a layman, or for that matter a politician, should require All the more welcome then, is a recent pamphlet *Offensive Missiles* by Kosta Tsipis of the Stockholm International Peace Research Institute (Stockholm Paper 5) The author tries to form numerical estimates of destruction capabilities of the US and Soviet land-based missiles and concludes that the United States has been, is and will continue to remain well ahead

As long as the accuracy of missiles was inadequate to guarantee that the opposition's missile silos would be wiped out by a pre-emptive strike, it was foolish for a potential aggressor to target missile bases extensively, since the counterpunch aimed at cities could be so massive Thus for many years targetting doctrine was centred on the destruction by both sides of cities Gradually, however, the acquisition of missiles with higher accuracies capable of splitting in flight into several re-entry vehicles (MIRVs) each with its own target has enabled the possibility to be reconsidered of taking out all silos at each 'farm' and all farms simultaneously But this operation depends crucially on accuracies and reliabilities being sufficient to ensure that few, if any, opposing missiles remain serviceable That this is now on the cards is clear from, among other things, the declaration of the US Secretary of Defence, Mr Schlesinger, of the US government's intention to go for a counterforce capability

Warheads can destroy missiles in their silos by a variety of methods the overpressure from a blast is perhaps the most damaging effect and consequently silos are often hardened to withstand pressures up to several hundred pounds per square inch Let H be the hardening pressure In dealing with destruction probabilities the yield in megatons (y) and the circular error probability (CEP) in nautical miles appear in the ratios $y^{\frac{2}{3}}/(CEP)^2$ which call K, the lethality

Then if n warheads are aimed at the same target, the probability of demolishing it with at least one warhead is

$$P=1-\exp\left\{\frac{Kn}{2H^{\frac{2}{3}}\{fH\}^{\frac{2}{3}}}\right\}$$

The function $f(H)$ is slow varying and for H in the range 100–1,000 pound per square inch is roughly 0 15 Thus, for example, to have a probability of 90% of destroying a silo hardened to 1,000 p s i a value of Kn of 71 is needed

Tsipis makes the important point that countersilo capability of a strategic nuclear force is thus dependent on the product Kn summed over all missiles This should allow us to calculate the relative abilities of the United States and Soviet Union to annihilate each other's missile forces The figure shows how the two sides stand in 1974

The weakest element in all this is knowledge of the accuracy of the re-entry vehicle as reflected quadratically in the CEP The US values are fairly easily inferred from the open literature, but can the Soviet values—in all cases inferior to those of the US—be relied upon? Tsipis follows a policy of minimising the difference between Soviet and US accuracies the Soviet value is the most optimistic, the US value the most pessimistic supportable by published figures This may be sound policy, but it will raise a few eyebrows that Soviet missiles are so inferior to

American ones Our only knowledge of Russian missile capability comes from US intelligence sources and this must surely be a suspect channel since there may be political reasons at any time to exaggerate or underplay opposition strength (only three years ago the same sources were erring by a factor of five in the relatively simple business of estimating the yield of Soviet nuclear tests) Since the value of *K* is so strongly dependent on *CEP* and since Tsipis is strangely unprepared either to substantiate his figures or to point out publicly the weakest link in the chain, it should be said that the figures consequent on *CEP* are one man's estimate

If however they are to be trusted the inference is fairly clearly that whether by number of missiles (though not launchers) or by *Kn*, the United States is at present in a comfortably superior position, although Tsipis goes on to show that the value of *Kn* required to destroy all silos of either country with 90% probability is still larger than present values (see figure), so there is not yet a counterforce capability on either side

Even if in the course of continuous improvement (including terminal guidance on to targets) either side gets the theoretical capability to demolish all the missile bases of the other side, there are reasons to believe that this could not be converted into an assured practical capability Tsipis points out that there are at least two unknowns, one technical and one strategic

The effect of one nuclear weapon detonating in the vicinity of a missile site is to make the delivery of a second a highly uncertain process in the period during which the fireball effects persist On the other hand this makes the launch of missiles also a speculative process An attacking nation must aim for a simultaneous attack on all silos in the farm with such precision that a second volley is unnecessary

In all these calculations the submarine fleet, which may have saved some of its missiles, remains inviolate, as the location and destruction of submarines is still a highly chancy process Thus either or both sides could then launch a salvo against cities from submarines, and bombers would also come into their own

Do we know that either side would be caught flat-footed by a first strike? It seems unlikely that the attacked would fail to launch his missiles immediately the intention became clear—and these would be aimed at cities Thus counterforce is a strategy that without extraordinary assurances would be extremely perilous, and (as Tsipis points out) only guarantees an ever-increasing expenditure on nuclear arms

One point must be made in conclusion SIPRI has been trusted over the past decade for its immensely impressive output of objective figures It has recently, in my opinion, started to use the figures to pass value judgements on various governments The trend is clear in the conclusion to the present booklet which contains gratuitous comments and guesses on the superpowers and their intentions This diminishes the value of the report I have already noted that the figures on accuracy are inevitably subjective once views are expressed in a paper on the "unjustified and unnecessary" nature of US requests and on the doubtfulness that the Soviet Union will display "rational restraint" it is difficult to know whether these views have, even unconsciously, fed back into the subjective estimates

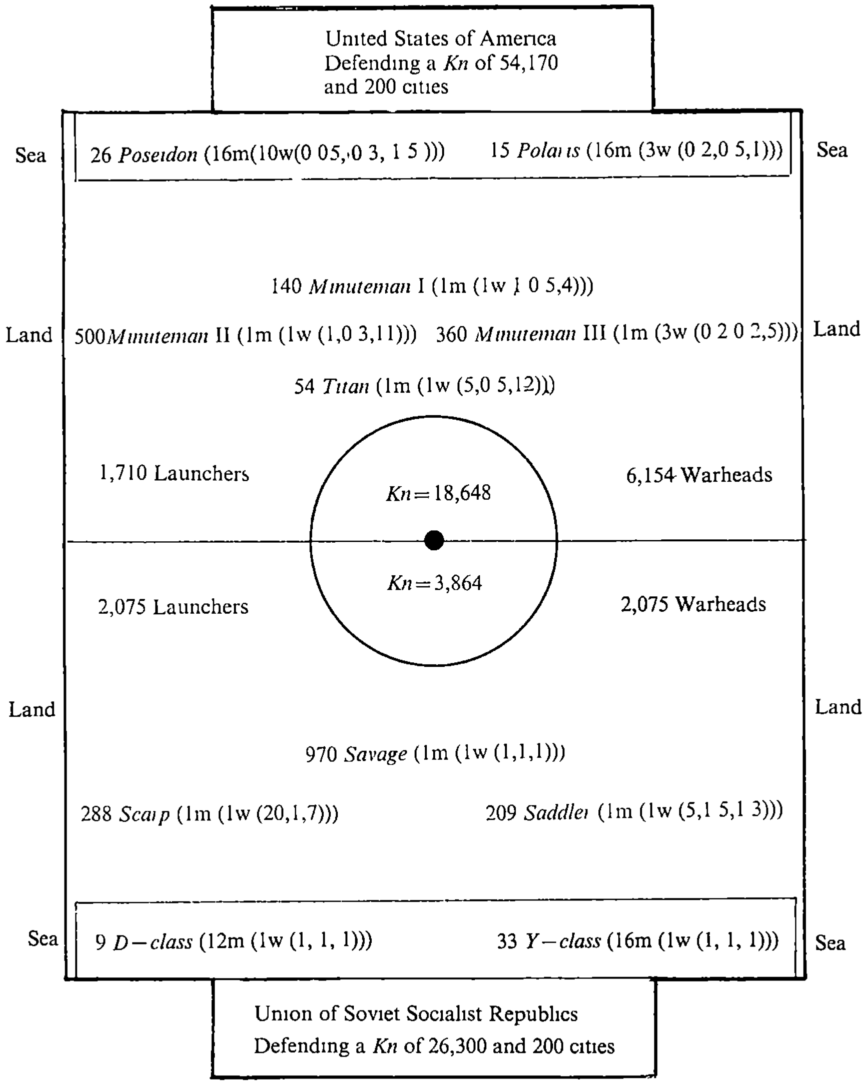
United States of America
Defending a *Kn* of 54,170
and 200 cities
Sea
26 *Poseidon* (16m(10w(0 05,0 3, 1 5)))
15 *Polaris* (16m (3w (0 2,0 5,1)))
Sea
140 *Minuteman* I (1m (1w 1 0 5,4)))
Land
500*Minuteman* II (1m (1w (1,0 3,11)))
360 *Minuteman* III (1m (3w (0 2 0 2,5)))
Land
54 *Titan* (1m (1w (5,0 5,12)))
1,710 Launchers
Kn = 18,648
6,154 Warheads
2,075 Launchers
Kn = 3,864
2,075 Warheads
Land
Land
970 *Savage* (1m (1w (1,1,1)))
288 *Scarp* (1m (1w (20,1,7)))
209 *Saddler* (1m (1w (5,1 5,1 3)))
Sea
9 *D*–*class* (12m (1w (1, 1, 1)))
33 *Y*–*class* (16m (1w (1, 1, 1)))
Sea
Union of Soviet Socialist Republics
Defending a *Kn* of 26,300 and 200 cities

Fig 1 How they line up 54 *Titan*(1m(1w(5,0 5,12))) means there are 54 *Titan* sites, each with one missile, each missile carrying one warhead, each warhead being of 5 megatons, with an estimated *CEP* of 0 5 nautical miles and a *Kn* of 12 The total *Kn* to destroy all US silos is 54,170 (at 90% probability), the Soviet Union defends a value of 26,300

Immunisation with steroids

from our
Steroid Biochemistry Correspondent

Much has been learnt about the biological role of hormones by surgical removal of the endocrine glands responsible for their secretion and administration of components of glandular secretions to determine the reversibility of the changes produced But many glands secrete more than one hormone and it is desirable to selectively prevent or neutralise the secretion of a single hormone Antibodies against specific secretions are now making this approach possible For example, immunising an animal to its own steroid hormones reduces the concentration of circulating non-protein-bound steroid, the biologically active fraction, and prevents its interaction with tissue receptors

Some studies with antibodies to the female sex hormones have already been reported in rats This approach has been extended by Nieschlag *et al* (*Acta endocr*, *Copenh*, **76**, 556–569, 1974) to study the role of various steroids in the control of testicular and adrenocortical function As might be expected immunisation of rabbits with an antiserum to testosterone induced hypertrophy and hyperfunction of the testis as determined by histometry and measurement of serum testosterone levels But the weight of the accessory reproductive glands of the testosterone-immunised rabbits was lower than that of control animals, the prostatic epithelium was atrophied and the diameter of the alveoli reduced showing neutralisation of the biological action of testosterone Elevated concentrations of luteinising hormone and follicle-stimulating hormone were detected in blood and the changes produced were similar to those induced by administration of these gonadotrophins This increased secretion of gonadotrophins from the pituitary seems to result from binding of the testosterone to the circulating antibody making it unavailable to the hypothalamic receptors

In animals immunised against cortisol but not those receiving testosterone, androstenedione or oestradiol there was evidence for hyperfunction of the adrenal cortex In rabbits immunised against oestradiol there was an increased secretion of luteinising hormone and Leydig cell hyperplasia of the testes similar to that observed after testosterone administration but in contrast there was no change in follicle-stimulating hormone levels, testicular weight or in accessory reproductive glands The findings suggest therefore that oestradiol may have a differential effect on gonadotrophin secretion in the rabbit The results of immunisation against other related steroids were more difficult to interpret because of some degree of cross reaction of the antibody with other hormones

This way of investigating the role of various steroids in feedback control systems and their interaction seems very promising It will no doubt be developed further in the future if more specific antibodies to a wide range of steroids can be developed

Plants which survived glaciation

from Peter D Moore

THE explanation of present day, disjunct distribution of plants and animals by reference to climatic changes during the Pleistocene is a process fraught with difficulties European biogeographers have continually been at loggerheads with their geological colleagues concerning the likelihood of certain species surviving in isolated pockets or on solitary peaks during periods of major glacial advance In Norway a number of species show a bicentric distribution having stations on the northern and on the southern ends of the Scandinavian mountain chain, but being absent in the central area (for example, *Campanula uniflora*) Per-glacial survival in these two areas has been invoked as an explanation (Nordhagen in *North Atlantic Biota and their History*, edit by A Love and D Love, 241, Macmillan, Toronto, 1963) Similar suggestions were put forward in the explanation of disjunct groups of arctic alpine plants in Britain, such as the Teesdale assemblage (see *Nature*, **249**, 798, 1974) In both British and Norwegian situations, however, the problem may well be one of post-glacial disjunction owing to the extent of forest cover during the climatic optimum The survival sites may be post-glacial rather than per-glacial refugia and may represent relict fragments of plant communities which were widespread at the close of the last glaciation

In North America, a similar problem exists in the Gulf of St Lawrence area, where the disjunct distribution of some plants has been explained as the product of survival through the Wisconsin glaciation, but once again post-glacial forest spread could have been the agent of disjunction In the west, parts of the Yukon have never been glaciated and are thus undisputed potential refugia for those species capable of survival under periglacial conditions (see Lindroth, *Endeavour*, **29**, 129, 1970) Packer and Vitt (*Can J Bot*, **52**, 1393, 1974) have now proposed that the Mountain Park area of the Canadian Rocky Mountains in Alberta may have served as a glacial refugium

Their argument is based mainly upon the collection of disjunct arctic-alpine and alpine plant species which are found together in this area, though they claim that geological evidence also supports the idea of an ice-free area existing throughout Wisconsin times The ice-free regions would have included some foothill areas as well as protruding mountain peaks (nunataks) The disjunct species involved include some, such as *Eriophorum callitrix*, with a mainly arctic distribution, some, such as the bryophyte *Orthotrichum pylaisii*, which occur in both arctic and alpine situations, and some, such as *Telesonix jamesii*, which are essentially alpines and occur in isolated mountain sites mainly to the south of Mountain Park A collection of disjunct species (the authors name sixteen), however, does not in itself prove per-glacial survival There are two alternative explanations which must be examined first, namely long-distance immigration from a distant centre of dispersal, and disjunction by forest invasion of territory once occupied by the relicts

Long-distance dispersal is unlikely to account for all the species, since most have very poor dispersal mechanisms If forest spread had caused the disjunction, then one would expect survival of at least some of the species at various sites between their now isolated stations, since at no time in the post-glacial period has the tree line been high enough to eliminate them from all geographically intermediate sites They are, however, absent from these The authors' hypothesis of *in situ* survival of the Wisconsin glaciation is thus the simplest available to explain the distribution

One must still ask why the plants did not spread from this centre during the conducive climatic conditions of late-Wisconsin times, prior to forest invasion It is difficult to conceive of severe genetic impoverishment occurring in populations isolated for a period (frequently interrupted) of at most 100,000 yr One must hope that future palaeoecological studies of those species will supply more information on their glacial histories Meanwhile, it is a matter for concern that this area is not included in any Canadian or provincial conservation programme and is currently threatened by mining

Plumbing at the North Pole

from Peter J Smith

ALTHOUGH the original soundings taken by Nansen (recorded in *Norwegian North Polar Expedition, 1893–96*, vol 4, Jacob Dybwad, 1904) left no doubt that the Arctic Ocean is a deep water basin, the full complexity of the region has only become evident since modern surveys and traverses began in the later 1950s Few would now disagree with Dawes (in *Implications of Continental Drift to the Earth Sciences*, Academic Press, 1973) when he says that "the basin is not a simple deep structure with uniform character but is made up of individual basins separated by submarine ridges of contrasting morphology", although there would perhaps be less agreement on how the ridges were formed and hence on what they represent in terms of plate tectonics

There are three ridges in the Arctic Ocean The Lomonosov ridge is flat-topped and relatively narrow, rises to within 954 m of sea level, extends 2,000 km from the vicinity of northern Greenland and Ellesmere Island to the New Siberian Islands off the coast of Russia, and splits the total basin unequally into the Amerasian (larger) and Eurasian (smaller) basins The Amerasian basin, in turn, is divided unequally by the Alpha ridge, a broad fractured structure with rugged topography and complex morphology, and the Eurasian basin is cut roughly in half by the Nansen ridge, a seismically active offset extension of the mid-Atlantic ridge Significantly, the three ridges are almost parallel to each other Less significantly, the Lomonosov ridge is unique in that its edge lies beneath the north geographical pole

All the evidence suggests that the Nansen ridge began spreading about 40 million years ago and continues to do so at a rate of about 1 0 cm a year, it has a clear geographical relationship with the mid-Atlantic ridge, is associated with a well defined pattern of earthquake epicentres, and gives rise to typical linear magnetic anomalies (albeit with smaller than usual amplitudes) The Alpha ridge, on the other hand, is evidently inactive in terms of both seismicity and spreading, although it is associated with linear magnetic anomalies of high amplitude This last observation led Vogt and Ostenso (*J Geophys Res*, **70**, 4925, 1970) to propose that the Alpha ridge, though now dormant, is the site of ancient seafloor spreading According to this plausible interpretation, spreading would have begun about 200 million years ago (possibly simultaneously with the opening of the North Atlantic) and ended about 40 million years ago when the activity transferred to the Nansen ridge Dawes tends to be more cautious about this, drawing attention to Vogt and Ostenso's own admission that the Alpha ridge may represent "something entirely different"

But if the Alpha and Nansen ridges are essentially oceanic features, the Lomonosov ridge is thought to be of continental origin, although it lies in an anomalous geographical position It is aseismic, has no linear magnetic anomalies associated with it and it is generally quiet magnetically—all of which suggests a scarcity of igneous rock But this is negative evidence, and positive evidence in favour of a continental composition is more sparse It is true that a seismic profile obtained by Ostenso (in *Implications of Continental Drift to the Earth Sciences,* Academic Press, 1973) indicates a smooth topography and a deep sedimentary structure, but again this relies for much of its effect on a comparison with known oceanic ridge structures It is also true that positive, if indirect, evidence for the Lomonosov ridge's continental nature may be adduced from structures on the land adjacent to the Arctic Ocean, although, as Dawes points out with respect to the alignment of the ridge with geological features on Ellesmere Island, this support could prove to be ambiguous in the face of data suggesting that the ridge, although continental, originates elsewhere

More positive evidence is thus urgently required—and has now been provided, within limits, by Lillestrand and Weber (*J Geophys Res*, **79**, 3347, 1974) During the Second Canadian North Pole Expedition (1969) these workers measured the plumb line deflection in the vicinity of the Lomonosov ridge near the North Pole by comparing the paths of a drifting ice island station as measured astronomically and as determined by means of a Transit Satellite receiver (The displacement between the two independently determined paths gives the plumb line deflection almost directly) The mean deflection at 89° 40′ N, 77° W was found to be 9 arc s towards 34° E, and the estimated deflection at the rotational north pole is 4–8 arc s in the direction 20–40° E

This is apparently the first time that the plumb line deflection at the North Pole has been determined, and so the result is of interest in itself But it is also important in connection with the nature of the Lomonosov ridge, for Lillestrand and Weber go on to show that the measured deflection is in good agreement with the value predicted from a model constructed on the assumption that the ridge is a sedimentary structure underlain by a lower density continental crustal block Moreover, several gravity measurements made in the area are consistent with the free air gravity anomaly computed from the same model. Of course, all gravity-based interpretations are ambiguous in that any data set can be accounted for by a large number of different structures Within this limitation, however, the new result from the North Pole cannot but strengthen support for the view, first put forward by Heezen and Ewing (in *Geology of the Arctic,* University of Toronto Press, 1961) and later expanded by Wilson (*Nature,* **198**, 929, 1963), that the Lomonosov ridge represents a section of the Siberian continental margin split off when the Nansen ridge became active beneath the edge of Eurasia

Democratic science

THE multiauthor paper has become a commonplace and the multinational project has come into its own But authors usually achieve a consensus before publishing and present a united if sometimes cautious front The latest multinational attempt to resolve some of the thorny problems of mycobacterial taxomy (Meissner *et al*, *J gen Microbiol*, **83**, 207, 1974), however, has resulted in a published majority report with six out of the twenty-two authors presenting their own dissident statement on one of the proposals Perhaps this approach could be encouraged, it would certainly make for more interesting reading in most scientific papers

Diseases of skeletal muscle

from a Correspondent

MUCH of the work of the Third International Congress on Muscle Diseases (Newcastle upon Tyne, September 15 to 20) dealt with the interaction between nerve and muscle Dr L Guth (National Institutes of Health, Bethesda) opened the meeting with a review of trophic interactions, dealing especially with cross innervation studies Other workers discussed experiments utilising chronic stimulation of nerve or muscle both in animals and man to alter the histochemical, biochemical and physiological characteristics of muscle

In a session on myasthenia gravis Dr E Heilbronn (Stockholm) described the production of experimental myasthenia gravis in rabbits immunised with a purified preparation of acetylcholine receptor from the electric organ of *Torpedo,* and the effect of antibodies against this receptor This will probably prove a fruitful model for the investigation of abnormalities of neuromuscular transmission Various other immunological aspects of myasthenia gravis were considered, including the significance of HL-A antigens and various skeletal muscle and other antibodies Dr G H Oosterhuis (Amsterdam) reported no correlation between thymic histology and the prognosis for myasthenia gravis

A session was devoted to the neural theory of muscular dystrophy Several workers, including Drs C P Panayiotopoulos (Athens National University), J P Ballantyne (University of Glasgow) and Professor W G Bradley (University of Newcastle upon Tyne) questioned the evidence upon which the theory was originally based They considered both technical aspects and questions of logic, Professor A J McComas (McMaster University) defended the theory Both Panayiotopoulos and Ballantyne reported studies using different techniques of motor unit counting in human muscle, finding no fall in the number of motor units in dystrophic patients compared with their own controls The estimated number by their two different techniques was however not identical Bradley reported that changes in the nerves of mice with muscular dystrophy were not found in patients with Duchenne muscular dystrophy, though Dr Mukoyama (Nagoya) described some changes of the myelin sheaths in the spinal roots of such patients Bradley's studies repeating the transplantation experiments originally taken to support the neural theory gave reason to suspect that the contraction of the host muscle might be recorded from the transplanted regenerate Dr Neerunjun (Royal Postgraduate Medical School) however reported studies which supported the neural theory Dr A C Peterson (University of British Columbia) described chimera experiments indicating that a skeletal muscle fibre might be morphologically normal though it had 96% dystrophic nuclei From these experiments he concluded that the cause of murine muscular dystrophy lay outside the skeletal muscle In discussion, the question was raised whether one or two per cent of normal nuclei might provide sufficient of a putative deficient substance to cure the disease Dr M Rathbone (McMaster University) showed that transplantation of foetal spinal cord from dystrophic chicks could produce biochemical enzyme changes indicative of dystrophy in otherwise normal chicks

A session was devoted to papers bearing on the vascular theory of muscular dystrophy Neither Drs O B Paulson and F Jerusalem (Mayo Clinic, Rochester) nor Dr M D O'Brien (University of Newcastle upon Tyne) found decreases in muscle blood flow or capillary abnormalities in Duchenne muscular dystrophy Professor K Kunze commented that denervated human skeletal muscle shows similar or even lower P_{O_2} values when locally measured and compared with those in Duchenne and other muscular dystrophies No defence of the vascular theory came in discussion from Dr W King Engel (National Institutes of Health, Bethesda), though he restated his theory in a later review session dealing with aetiological theories

In sessions on the clinical management of human muscular dystrophy Dr I M Siegel (University of Chicago) reviewed the results of his enthusiastic and effective treatment of muscular dystrophy with surgery and surgical appliances Dr R H T Edwards (Royal Postgraduate Medical School) described a simple dynamometer for use in clinical studies of patients with muscle weakness Dr J B Peter (University of California, Los Angeles) described a controlled trial of long-term high dose corticosteroid therapy in Duchenne muscular dystrophy Initial results indicated a fall in the serum enzyme activities, but no certain clinical improvement to date

Other sessions dealt with normal muscle, with various forms of myopathy, malignant hyperpyrexia, and myositis, as well as the various animal models of muscular dystrophy Biochemical studies of membrane abnormalities in myotonic conditions proved stimulating Drs A D Roses and S H Appel (Duke University) reported various membrane abnormalities, including that of membrane protein phosphorylation in dystrophia myotonica Dr R M Andiman (University of California, Los Angeles) found an increase of desmosterol in the membranes of animals rendered myotonic by various drugs, but no increase in human myotonic membranes

Peripheral nerve studies in health and disease were fully considered in the congress A session and a workshop were devoted to axoplasmic flow Dr S Ochs (University of Indianapolis) reviewed the evidence for fast and slow rates of axoplasmic flow and Dr G W Gross (Munich) described his experiments on the garfish olfactory nerve, which led him to propound a theoretical model of the many features making up axoplasmic flow Dr B G Livett (Monash University) (for Professor L Austin) described experiments showing marked abnormalities of axoplasmic flow of proteins and lipids in the nerves of dystrophic mice In a fruitful workshop on motor unit function, Professor E Henneman (Harvard) described evidence that motor units have a regular and repeatable order of activation This gave rise to considerable discussion, not all speakers being convinced by the evidence

Mad March hares

from our Animal Ecology Correspondent

THE springtime behaviour of hares, normally peaceful and retiring creatures, is proverbial During February, March and April they gang together, becoming bellicose, vocal and extrovert The 'madness' lasts only a few weeks, beginning at the start of the breeding season but ending long before the breeding season is finished

In a thorough study on the breeding biology of the European hare, G A Lincoln has brought to light some intriguing questions (*J Zool, Lond*, **174**, 1, 1974) The period of sexual quiescence in does lasts from September to January, and in bucks viable sperm occurs in the epididymides from mid-December to early September The number of sperms present reaches a high level from February to June with the maximum testosterone content in April Pregnancies were recorded every month from January to August with the maximum number of females pregnant and the highest mean number of foetuses per pregnancy occurring in April, May and June

Lincoln found spermatozoa in cervical smears taken from does during November and December, well before the first ovulation of the season (In this mammal ovulation is initiated by coitus Stieve, *Zool Anz*, **148**, 101, 1952) The hormonal complexion of the hare does not allow ovulation to occur until early January, so matings earlier than this fail to result in pregnancy Perhaps such matings have a social function providing necessary courtship and mating experience for young animals

'March madness' is thought by Lincoln to be a rut having the function of stimulating the resumption of breeding activity among the does Wynne-Edwards, however, believes it to be a form of self advertisement (*Animal Dispersion in Relation to Social Behaviour*, Oliver and Boyd, Edinburgh, 1962) The complex chasing and boxing of the 'madness' is a social means of establishing a breeding hierarchy amongst the males, possibly identifying socially inexperienced individuals from the rest He argues that this must happen right at the start of the breeding season so that the year's "quota" of reproduction can be assessed Both interpretations can be correct and verification must await further study Certainly the behaviour has little immediate physiological effect as only 16% of March madness conceptions result in hares the following spring compared with between 39% and 45% from later conceptions

The suggestion that a mechanism exists whereby young hares gain sexual experience before actually breeding has a parallel in at least one other species of mammal Young male baboons mate with females at the very start of their oestrus cycles when the females' receptivity and chance of pregnancy is low Towards the end of their cycles, females mate with older and more experienced males when their receptivity is high Most pregnancies result from these late matings (Washburn and DeVore, *Scient Am*, June 1961) Perhaps even hares have a society with complex rules of conduct which offers to instruct young initiates in its mysteries

articles

Chemical uniformity of airborne particulate material, and a maritime effect

D. H. Peirson, P. A. Cawse & R. S. Cambray

Atomic Energy Research Establishment, Harwell, Oxfordshire, UK

Airborne particulate material has a uniform elemental composition, in which the industrial elements are enriched relative to soil In the surface of the North Sea the trace element content seems to be massively enhanced relative to bulk seawater

DURING 1972–3 measurements of the trace element content of airborne dust, rainwater and the dry deposit, sampled at various places in Britain, following exploratory work at a single location[1,2], has yielded much basic information on the annual deposition inventory, the "washout factor", and the "dry deposition velocity" which will be described in detail elsewhere[3,4] Meanwhile it is opportune to report observations that, first, demonstrate a remarkable uniformity in the elemental composition of the general aerosol and, second, suggest a considerable enhancement of the trace element content of the ocean surface

The methods of sampling and analysis have been published previously[2] and will be restated only briefly Samples of airborne dust have been collected by drawing air through a filter paper (Whatman 40) held in a polypropylene duct, which is capable in still air of collecting particles (specific gravity 1) of diameter up to about 150 μm Rainwater is sampled by polythene funnel and bottle Because the funnel is exposed continuously, both wet and dry deposition are collected The dry deposition is collected separately on a horizontal sheet of filter paper (Whatman 541) sheltered from direct rain Samplers are mounted at 1 5 m above ground, are run continuously and are changed each month The location of the sampling stations is shown in Fig 1

Samples are analysed mainly by neutron activation followed by gamma-ray spectrometry but also by X-ray fluorescence, atomic absorption and chemical methods

Chemical uniformity

The average concentrations in air during 1972 of about 30 trace and other elements at seven sites in Britain are tabulated in Table 1 The sites were chosen to represent rural and more industrial cases, although all are non-urban The range of the values for an (non-maritime) element seem to span an order of magnitude or more Now if the results from each site in Table 1 are referred to a single element, say scandium, then the ratios so obtained show much less variation from place to place (except for Na, Cl and Br) If in addition the concentration ratio of an element relative to scandium is normalised to the same ratio given by Bowen[5] in average soil then this "enrichment factor" shown in Fig 2 demonstrates the reduction in variation from place to place It also separates the elements into two classes, those that are "soil-derived" like scandium having factors about unity and those of "industrial" origin that are enriched relative to soil by a few to many times There are two anomalies in Fig 2—the large excesses of cobalt and nickel at Trebanos are probably due to nearby industrial sources Obviously it is not difficult to seek excessive and anomalous values, for example, in a busy street in London during July 1972 the enrichment factor for lead was nearly 8,000 whereas the general rule from Fig 2 is that the ratio of lead to scandium is sensibly constant over all the sites at a value somewhat more than 1,000 times that in nominally average soil Enrichment factors may be derived from observations at more distant sites in the northern hemisphere For example, elemental

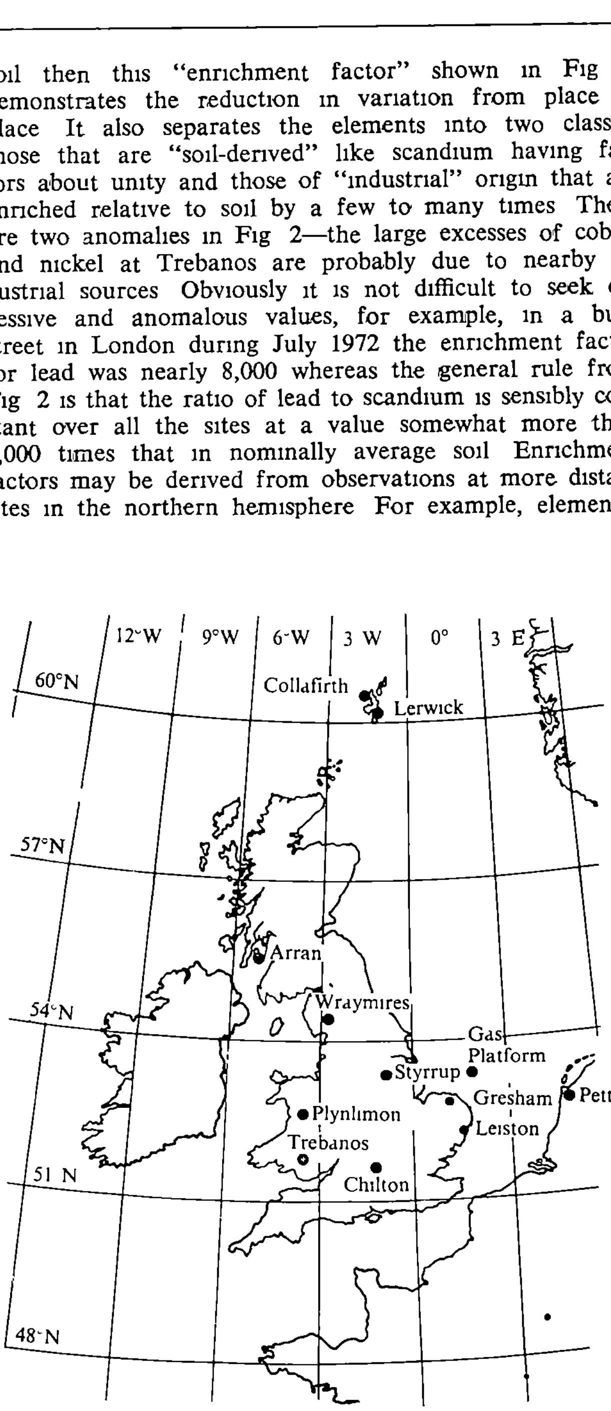

Fig 1 Location of sampling stations

Table 1 Elemental concentrations in air (ng per kg air) January–December 1972

Element	Chilton Berks	*Leiston Suffolk	Lerwick Shetland Is	Plynlimon Montgoms	Styrrup Notts	Trebanos Glamorgs	Wraymires Lancs	Average soil (ref 5) (p p m)
Na	760	990	1,900	800	900	1,000	540	6,300
Al	270	230	58	115	640	280	96	71,000
Cl	2,100	2,000	3,100	1,350	4,100	2,300	1,050	100
Ca	<610	<410	<550	<570	<1,100	<550	<260	13,700
Sc	0 065	0 058	0 014	0 029	0 18	0 102	0 029	7
V	14	11	2 3	4 2	21	14	5 0	100
Cr	4 4	7 5	1 1	2 0	17	9 6	1 5	100
Mn	20	20	3 1	9 0	47	18	7 8	850
Fe	310	340	67	140	940	430	140	38,000
Co	0 35	0 37	0 066	0 20	0 76	8 5	0 11	8
Ni	4 5	4 1	2 0	3 1	8 8	66	2 8	40
Cu	13	<9	<3	11	34	29	<6	20
Zn	155	160	39	76	340	190	56	50
As	5 1	4 9	1 9	2 0	24	7 0	2 7	6
Se	1 2	1 3	0 41	0 44	3 2	1 8	0 52	0 2
Br	39	31	13	11	130	40	16	5
Rb	<4	<3	<0 6	<1	<12	<8	<1	100
Cd	<27	<11	<7	<7	<34	<22	<16	0 06
In	<0 4	<0 3	<0 1	<0 2	<0 9	<1	<0 1	—
Sb	2 1	2 8	0 51	0 72	5 9	2 1	1 2	~6
I	<2	<1	<0 7	<2	<4	<4	<2	5
Cs	0 23	0 31	0 05	0 09	0 59	0 31	0 10	6
La	<0 3	<0 1	<0 3	<0 2	<0 6	<0 7	<0 7	30
Ce	0 36	0 32	0 07	0 14	0 70	0 50	0 15	50
W	<0 6	<0 5	<0 6	<0 3	<2	<0 9	<0 9	1
Au	<0 02	<0 01	<0 01	<0 02	<0 08	<0 04	<0 01	—
Hg	<0 3	<0 2	<0 04	<0 06	<0 4	<0 4	<0 1	0 03
Pb	130	110	29	43	330	190	56	10
Th	0 068	0 046	0 026	<0 02	0 12	<0 1	0 017	5

* February–December 1972
No adjustments have been made for filter efficiency[2]

analysis of air samples from the Osaka district of Japan[6] can be summarised to give factors close to those reported in Fig 2 for V, Co, Ni, Zn, As, Se, Sb and Pb Another comparison using aerosol analyses by Rahn *et al*[7] from a remote site in northern Norway again illustrates the uniformity of enrichment factors for V, Cr, Zn, As, Se and Sb In fact Rahn (private communication) by considering the distribution of enrichment factors worldwide, from remote to industrialised areas, has also demonstrated a general uniformity but with a sharp rise of enrichment factor at the highest elemental concentrations, that is, in the vicinity of a source

The division of elements in Fig 2 into soil-derived of low enrichment and industrial of higher enrichment conforms very well to the classification performed previously[1,2] according to dry deposition velocity[8], that seems to be correlated with particle size The decrease of enrichment factor with increasing dry deposition velocity (rate of dry deposition/concentration in air) is shown in Fig 3 for trace elements determined at Chilton There is as yet no direct comparison at this site of particle size against deposition velocity However preliminary measurements[3] of the mass-median diameter at Chilton, using an Andersen sampler, confirm that deposition velocity increases with particle size and suggest that, at this site, a deposition velocity of 0 5 cm s^{-1} corresponds to a mass median diameter of about 1 μm (specific gravity 1) This suggests that those trace elements that are enriched above the

Table 2 Elemental concentrations in air (ng per kg air) June 1972–May 1973

Element	Leiston Suffolk	Gresham Norfolk	Collafirth Shetland Is	Petten* N Holland	Arran Bute	Lerwick Shetland Is	Gas platform N Sea
Na	850	1,050	1,700	1,500	1,400	1,600	3,300
Al	210	240	44	155	130	44	155
Cl	2,000	2,500	2,700	2,600	2,400	2,600	5,700
Sc	0 054	0 065	0 016	0 042	0 038	0 010	0 067
V	11 0	10 0	1 75	9 0	8 8	1 85	9 7
Cr	5 4	3 4	1 05	3 1	2 8	0 67	3 7
Mn	19 5	21	3 2	14 5	9 6	2 5	17 5
Fe	310	320	70	290	220	49	270
Co	0 33	0 31	0 21	0 23	0 27	0 051	0 29
Ni	4 8	5 4	2 9	7 3	4 7	2 7	8 2
Cu	<11	<9	<4	<6	<5	<8	<17
Zn	120	105	25	75	105	24	125
As	5 2	5 2	1 20	3 7	1 85	1 30	4 2
Se	1 30	1 40	0 43	0 96	0 87	0 30	1 20
Br	74	69	19 0	60	33	20	73
Rb	3 1	3 1	<0 5	<3	<2	<0 4	3 3
Sb	2 9	2 4	0 49	2 5	1 30	0 38	2 4
Cs	0 25	0.21	0 037	0 155	0 115	0 026	0 17
Ce	0 30	0 29	0 077	0 175	0 195	0 054	0 20
Pb	130	130	31	96	71	23	120
Th	0 042	0 053	0 012	0 032	0 030	0 013	<0 04

* February–May 1973 only
No adjustments have been made for filter efficiency[2]

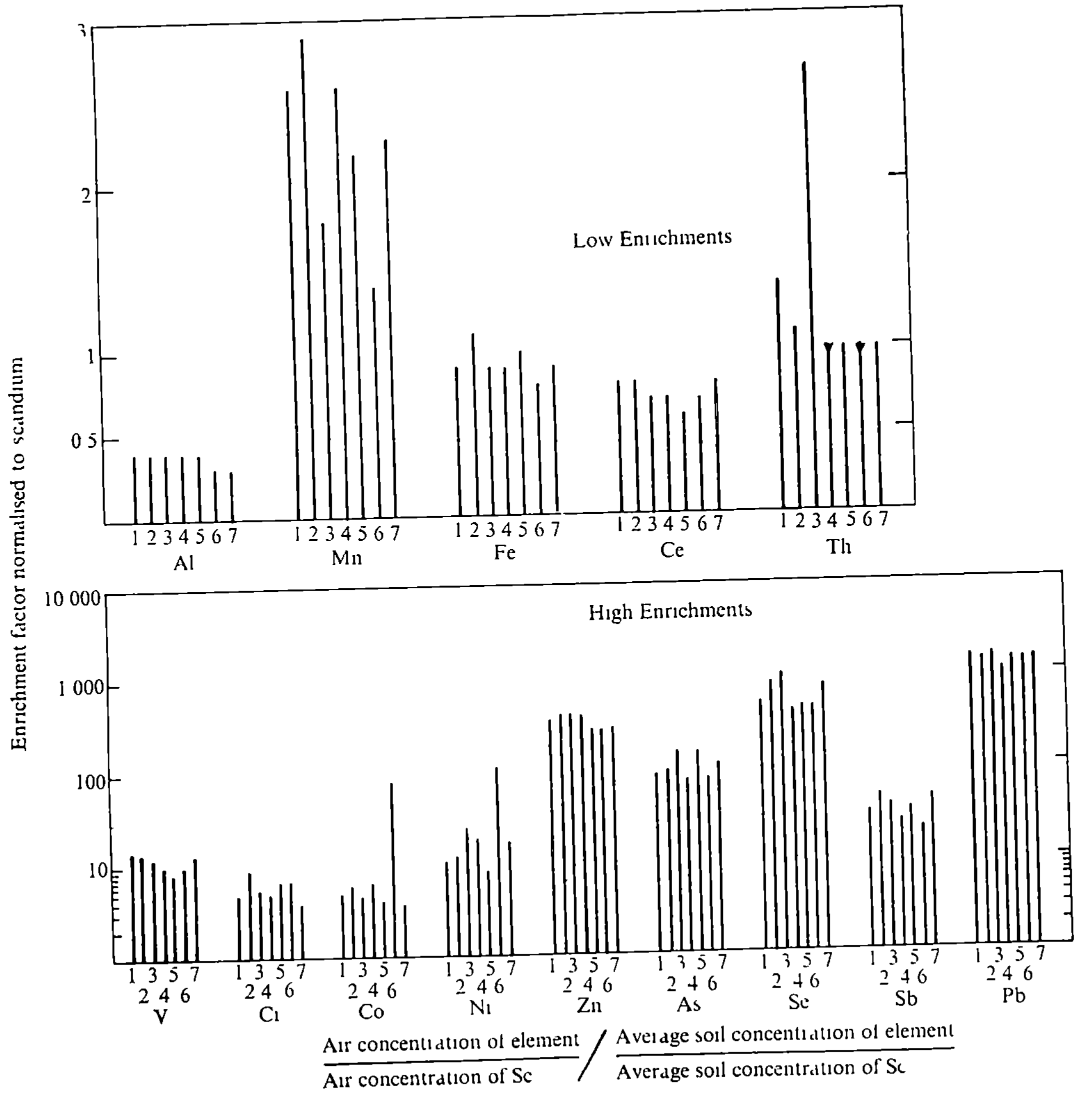

Fig. 2 Air particulate enrichment factors (1972) Sites 1 Chilton, Berks, 2 Leiston, Suffolk, 3 Lerwick, Shetland Is, 4 Plynlimon, Montgomery, 5 Styrrup, Notts, 6 Trebanos, Glamorgan, 7 Wraymires, Lancs

natural levels in soil appear as small particles, presumably formed by condensation after industrial combustion whereas natural particles formed by grinding and wind erosion are larger But it is not impossible that the combustion of fossil fuels for example can produce both large and small particles

If the results of the measurements presented above are representative not only of Britain but also of foreign sites in the same latitudes, then it is necessary to imagine and explain a general aerosol pervading this zone of latitude having a varying concentration but essentially the same elemental composition (except for the maritime elements Na, Cl and Br and also for regions in the vicinity of pollution sources, for example Trebanos, Fig 2) It is difficult to postulate a mixture of such sustained uniformity from separate natural and industrial sources that generate particles of differing characteristic sizes Material, that may be raised from the Earth's surface either by wind causing the suspension of soil and dust or by industrial processes, will probably lose particles of size greater than about 10 μm to gravitation It is possible that enough of the particulate material below this limit will diffuse vertically upwards and join the westerly airstreams in the middle and upper troposphere at altitudes up to about 15 km These air streams have been invoked to explain the passage of radioactive debris from nuclear explosions[9,10] Scavenging by rain will occur at and below cloud level (up to 3 km) and there will be continuous exchange by vertical diffusion throughout the troposphere There is supporting evidence in the long range transport of soils[11,12] Delany *et al*[13] measured vertical profiles up to an altitude of 9 km over continents and oceans, and found that over both, the component of the aerosol derived from the continents was dominant above about 2 km It is reasonable to relate the aerosol behaviour described above to the "tropospheric background aerosol" proposed by Junge[14]

There is thus a plausible explanation of the transfer of particulates from the surface and subsequent transport within the atmosphere, but not of the initial formation and subsequent maintenance of the mixture of elements It is suggested, but cannot yet be proved, that the natural and artificial aerosols are produced by the separate processes, are mixed in the atmosphere, are circulated zonally and eventually reappear at the Earth's surface Alternatively the aerosol mixture, or the part of it that is available for transport on a zonal or global scale, might be produced by a common process, not yet identified After deposition the dust is available for resuspension, more so from the paved surfaces of urban areas which are thereby exposed to higher concentrations of airborne dust than are rural areas

Provided that the observed effect of chemical uniformity is real and is not caused by some undetected artefact of the sampling equipment, then two points of significance emerge First, a scientific explanation of the formation of the aerosol mixture is required and could be pursued by a microscopic examination of the physical and chemical characteristics of the particles Second, in relation to the monitoring of dust-laden environments, it should

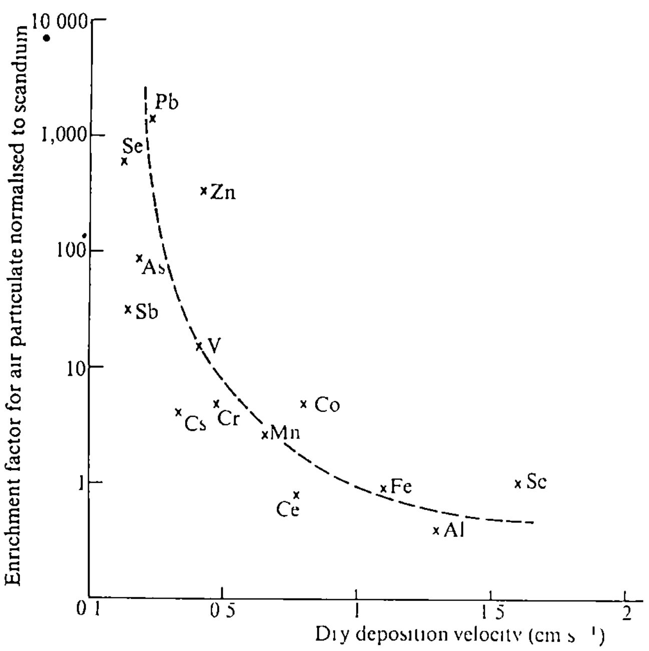

Fig. 3 Enrichment factor and dry deposition velocity at Chilton (Berks) 1972

be possible by comparison with the "normal" elemental mixture to identify and define the presence of elemental "pollution"

Maritime effect

The observations of the effect of the ocean upon the atmospheric aerosol are less direct and comprehensive than in the case given above The observations were made as part of a preliminary attempt to estimate the input of trace elements from the atmosphere into the North Sea[4]

The average concentrations of trace elements in air and rain for a 12-month period during 1972–3 are shown in Tables 2 and 3 The sampling stations were sited at or near the east coast of Britain except for Arran in the west of Scotland, a platform at 25 m above the North Sea about 55 km from the Norfolk coast and Petten in north Holland The only noticeable trends in the air concentration results of Table 2 are that the values at Lerwick and Collafirth, being clean and rural, are generally lower than elsewhere except for the maritime elements Na and Cl On the other hand the rain concentrations in Table 3 are higher by up to an order of magnitude at the platform in the North Sea than at the other sites, not only in the maritime elements but also in the other elements considered above to have soil and industrial origins Such an enhancement would complicate any attempt to calculate the deposition of trace elements by rain into the North Sea

There is no ready explanation of this effect The high values of elemental concentration in rain obtained at the platform are general it is unlikely that local contamination would elevate the values for all elements Only Zn appears in excessive amounts and is a possible contaminant from the platform itself Generally rain collectors situated on or near the sea would receive an amount of sea-spray that is significant compared with the actual rain that is collected As has been demonstrated by Freudenthal[15] for Sr-90, it is unlikely that this spray will scavenge trace elements from the atmosphere so much more efficiently than rain

The most plausible explanation is that the excess of trace elements in rain has arrived with the sea-spray, driven by wind from the ocean surface The air samplers do not register this excess presumably because the size of the spray particles is beyond the upper limit of collection of the sampling duct The limit calculated for the downward-facing duct would be reduced in winds having large horizontal velocities

The enhancement of the rain content by this maritime effect may be demonstrated by considering the concentration of an element in rain relative to that in air from Tables 2 and 3 This ratio ($\mu g\ l^{-1}$ of rain $\mu g\ kg^{-1}$ of air), which takes account of the differences from place to place in the concentration in air, is equivalent to the washout factor used previously[1,2,8] The relation between this rain air ratio and the seawater content of rain from site to site is demonstrated in Fig 4 for several elements With increasing seawater content, as indicated by the concentration of Na, the value of the ratio increases, typically by five times in the samples taken on the platform This

Table 3 Elemental concentrations in rain (µg l^{-1}) June 1972–May 1973

Element	Leiston Suffolk	Gresham Norfolk	Collafirth Shetland Is	Petten* N Holland	Arran Bute	Lerwick Shetland Is	Gas platform N Sea	Seawater	
								General (ref 16)	North Sea (ref 17)
Na	1,700	5,900	22,000	25,000	42,000	56,000	100,000	1 06×10⁷	
Al	360	630	<230	650	380	770	2,400	1 0–10	
Cl	3,000	12,000	46,000	45,000	93,000	105,000	210,000	1 9×10⁷	
Sc	0 076	0 092	0 021	0 110	0 097	0 092	0 49	0 04	
V	4 4	9 0	<8	8 9	<7	<6	<40	2 0	
Cr	4 8	5 0	1 85	2 6	3 1	3 1	20	0 13–0 25	
Mn	12 0	21	<5	23	<17	<24	<60		0 24–0 54
Fe	370	440	135	570	540	420	3,100		0 06–1 5
Co	0 55	0 63	0 190	0 68	0 41	0 32	3 2	0 2–0 7	
Ni	6 4	6 8	3 8	7 2	6 0	5 4	23		0 16–0 51
Cu	28	27	23	41	27	23	82		0 24–1 70
Zn	260	210	26	150	130	110	2,000		1 5–6 9
As	1 25	4 7	<8	12 0	<6	<17	45	3 0	
Se	0 48	0 69	0 40	0 46	0 39	0 91	1 75	4 0–6 0	
Br	33	130	360	390	460	1,300	2,300	6 5×10⁴	
Rb	<8	<8	<1 8	<7	<4	<6	<60	120	
Sb	1 20	1 10	0 30	2 0	0 46	0 67	5 9	0 5	
Cs	0 190	0 170	<0 03	0 185	0 080	0 092	1 70	0 5	
Ce	0 53	0 53	<0 1	0 55	0 43	<0 5	<4	0 4	
Pb	40	36	11 0	53	31	28	190		<0 05—0 8
Th	<0 08	<0 10	<0 07	<0 11	0 21	0 135	<0 7	0 5	
Rainfall mm yr⁻¹	417	476	1,542	516	1,129	1,201	260		

* February–May 1973 only

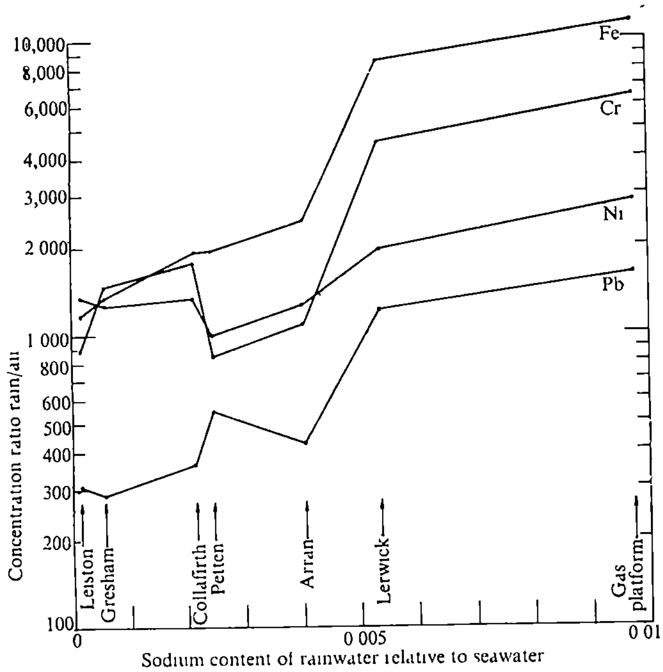

Fig 4 Elemental concentration ratio, rain/air and seawater content of rain (1972–1973)

implies that most of the elemental material measured in the platform samples has been collected as spray from the surface of the sea If the trace element content of these samples is compared with that in bulk seawater[16,17] (Table 3) relative to Na, then it can be seen that the concentration of these trace elements, derived from the sea surface, is enhanced by several orders of magnitude compared with that in bulk seawater

Duce *et al*[18] and Piotrowicz *et al*[19] have measured enrichments of up to 50 times in surface layers The present observation is consistent with their results extrapolated to the surface microlayer and also with the results of Szekielda[20]

An excess of material on the ocean surface can arise in two ways, either by deposition from the atmosphere or by concentration of material from within the sea The present work requires extension and elaboration to confirm these results, but if the enrichment of the sea surface is a real effect then it would be logical to investigate the mechanism of enrichment and also the role of the ocean as a source of material for transport in the atmosphere, locally and on a much larger scale

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Pitcairn Island—another Pacific hot spot?

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The volcanic Pitcairn Island, which has surface lavas with apparent ages between 0 45 and 0 93 Myr, may be the youngest member of another NW–SE trending volcanic lineament in the Pacific Ocean

PITCAIRN Island, in the south-eastern Pacific Ocean (24°04′S, 130°06′W), is built entirely of volcanic rock[1] Unlike the majority of volcanic islands in the Pacific basin, which are grouped in distinctive linear chains (see Fig 1), Pitcairn is isolated from its nearest neighbours, Oeno, Ducie and Henderson (all coralline islands) by 160 km, and it is 600 km from its closest volcanic neighbour, Mangareva in the Gambier Islands Easter Island lies approximately 2,100 km to the east, near the summit of the East Pacific Rise The position and youth of Pitcairn Island beg explanation by one or another of the rapidly proliferating theories of nonorogenic volcanism.

Pitcairn Island is built up from the seafloor, a depth of at least 3,500 m (ref 2) The age of the oceanic crust on which

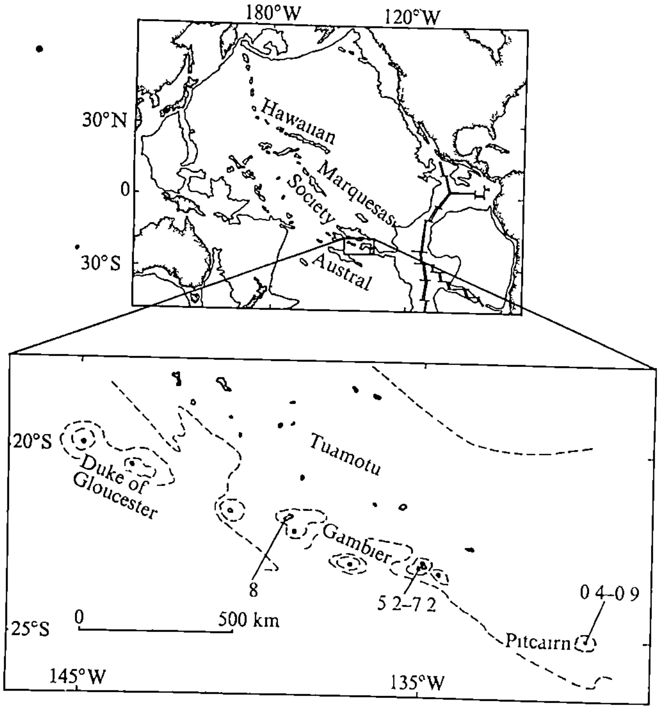

Fig. 1 Pitcairn Island is situated in the Pacific Basin, possibly the SE end of the Duke of Gloucester–Gambier Islands volcanic lineament which is parallel to other well known Pacific Island chains (Hawaiian, Marquesan, Society and Austral) The Duke of Gloucester Islands are all coral atolls at sealevel Numbers indicate K–Ar ages × 10^6 yr Bathymetry in the Pacific Basin denoted by the 4,000 m contour, in the inset by the 4,000 m and 2,000 m contours (dotted lines)

the island is constructed is not known with any great precision but magnetic anomaly studies[3] predict an age close to 30 Myr (anomalies 7–9) Basal sediments from Deep Sea Drilling Project (DSDP) site 75, 1,200 km NNW of Pitcairn (and roughly the same distance from the East Pacific Rise), have been assigned a lower Oligocene age (35–40 Myr)[4]

The island is approximately elliptical, measuring 4 km by 2 km and is cliffed on all sides, rising to an altitude of 347 m (Fig 2) Volcanic rocks of two types—lavas and pyroclastics—predominate Structurally, the island seems to be the remna of a single shield volcano, its dissected caldera rim forming prominent annular ridge which opens to the north Four uni have been discriminated the Tedside Volcanics—gentl dipping flows exposed near the west coast, the Christians Ca Formation—an agglomeratic tuff generally overlaying th Tedside Volcanics with erosional unconformity, the overlyin Adamstown Volcanics—horizontal flows which fill the centr basin, and the Pulawana Volcanics—a sequence of lava flow at the western extremity of the island, which may be coeva with the Adamstown Volcanics, as their lithology suggests There are also some dykes, mainly within the Tedsid Volcanics

Petrologically, the lavas range from alkali olivine basalt through hawaiites and mugearites to minor trachytes Th basalts contain phenocrysts of plagioclase feldspar, olivine an augite The hawaiites and mugearites exhibit trachytic textur and contain fewer phenocrysts than the basalts A commo additional groundmass phase in these samples is biotite The alkaline character of the trachytes is evident in phenocrysts o alkali feldspar, aegirine augite and fayalitic olivine Almost all of the rocks contain interstitial alkali feldspar, the amount increasing in the sequence olivine basalt to trachyte Chemical analyses of hawaiites, mugearites and trachytes from Pitcairn Island have been presented by Lacroix[5] who finds them comparable to the petrological association in the Marquesas Islands[5,6] New analyses from this Pitcairn collection will be published elsewhere

Geochronology

The samples selected for dating in this study are primarily hawaiites and mugearites All four lithological units are represented in the age determinations

Of the 17 selected samples, 12 were chosen for K–Ar age determination, on the basis of thin section examination Only those specimens which were devoid of any alteration of the K-bearing phases were selected Generally, they were holocrystalline and nonvesicular

Measurements were taken following the techniques previously described by McDougall[7–9] Constants used in the calculations are $\lambda_e = 0\ 585 \times 10^{-10}$ yr^{-1}, $\lambda_\beta = 4\ 72 \times 10^{-10}$ yr^{-1}, ^{40}K/K $= 1\ 19 \times 10^{-2}$ atom %

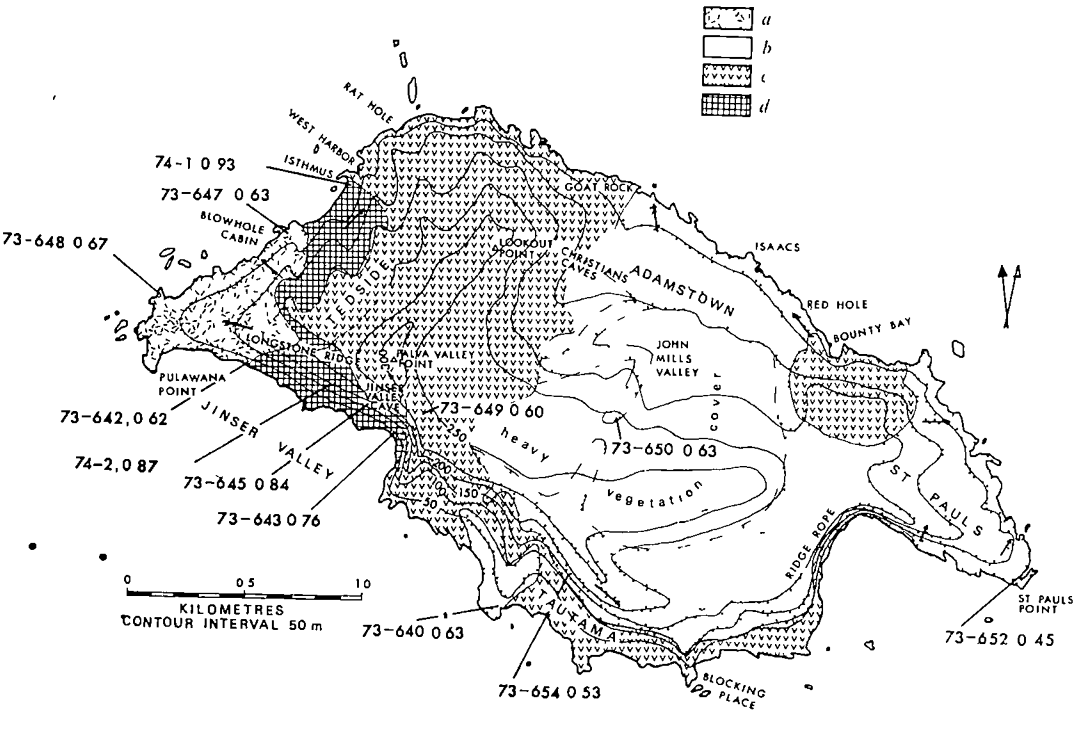

Fig 2 Generalised geology of Pitcairn Island (after Carter[1]) *a*, Pulawana Volcanics, *b*, Adamstown Volcanics, *c*, Christian Cave Formation, *d*, Tedside Volcanics Arrows indicate the generalised direction of lava flows Observed geological contacts indicated by solid line, inferred contacts, dashed line Numbers after commas, K–Ar ages (× 10^6 yr) of samples indicated Contours at 50 m intervals

Table 1 K–Ar ages on whole rock samples from Pitcairn Island

Laboratory No	Otago University No	Rock Type	K (weight %)	rad ^{40}Ar 10^{-8} cm^3 NTP g^{-1}	$\frac{100 \text{ rad } ^{40}\text{Ar}}{\text{Total } ^{40}\text{Ar}}$	Calculated age ± 2 s d (Myr)	Approximate altitude (m)	Locality
Pulawana Volcanics								
73–647	18815	Hawaiite	1 694, 1 696	4 27	32 5	0 63±0 01	25	Cabin Cave, NW coast
73–642	18740	Hawaiite	2 183, 2 169	5 42	28 8	0 62±0 01	10	Jinser Valley, SW coast
				5 41	28 9	0 62±0 02		
73–648	18823	Trachyte	3 826, 3 828	10 28	34 8	0 67±0 01	10	Dyke, W coast
Adamstown Volcanics								
73–652	18878	Hawaiite	1 537, 1 535	2 80	20 8	0 46±0 01	30	St Pauls Point, Eastern tip of island
				2 78	16 7	0 45±0 02		
73–654	18888	Biotite mugearite	1 718, 1 736	3 75	9 0	0 54±0 04	110	Artefact site, Tautama, adjacent to south coast
				3 61	18 0	0 52±0 02		
73–650	18825	Olivine mugearite	2 315, 2 311	5 63	26 0	0 61±0 02	200	Western side of John Mills Valley, centre of island
				5 96	36 7	0 64±0 02		
73–640	18730A	Mugearite	2 024, 2 036	5 11	31 7	0 63±0 01	20	Western end Tautama, southern coast, possibly Adamstown Volcanics
Christians Cave Formation								
73–649	18824	Hawaiite	1 274, 1 286	3 04	19 5	0 60±0 02	270	Block in agglomerate, Palva Ridge
Tedside Volcanics								
74–1	18755	Basalt	1 429, 1 418	5 41	40 5	0 95±0 02	10	Water Valley
				5 23	38 3	0 92±0 02		
74–2	18731	Hawaiite	1 960, 1 964	7 03	16 2	0 90±0 03	150	Jinser Valley
				6 65	51 3	0 85±0 01		
73–645	18764	Hawaiite	1 491, 1 496	5 01	46 0	0 84±0 01	130	Jinser Valley
				5 01	47 3	0 84±0 01		
73–643	18753	Basalt	1 065, 1 067	3 23	42 1	0 76±0 01	30	Jinser Valley
				3 28	34 5	0 77±0 02		

Table 1 presents the analytical data for 20 K–Ar age determinations on the 12 whole rock samples Generally, the duplicate analyses on 8 of the samples show agreement to within experimental error The samples are grouped according to the formations defined by Carter[1] Localities and ages are shown in Fig 2

The calculated ages fall into at least two groups Ages for four samples from the Tedside Volcanics, stratigraphically the oldest unit exposed on Pitcairn, form a group distinctly older than the rest, averaging 0 85 Myr and spanning the period 0 76–0 93 Myr The oldest apparent age was obtained on sample 74–1 from the north-west coast (near sealevel), about 1 km from the other three Tedside collecting localities which were in Jinser Valley The three Jinser Valley samples are from nearly horizontal lavas Samples 74–2 and 73–645 are from approximately the same stratigraphical horizon and give nearly identical ages, averaging 0 86 Myr The third sample (73–643), stratigraphically lower in the sequence, yields a significantly younger age of 0 76 ± 0 01 Myr (Because minor intersertal glass occurs in this sample it is possible that some radiogenic argon has been lost from it, resulting in a younger apparent age) These data suggest that much of the exposed Tedside Volcanics were erupted over a short interval of time between about 0 85 Myr and 0 93 Myr ago

The samples from the Christians Cave Formation, and the Adamstown and Pulawana Volcanics all yield younger K–Ar ages—from 0 46–0 63 Myr—consistent with their stratigraphically younger age A block from an agglomerate of the Christians Cave Formation yields an age of 0 60 Myr, indistinguishable from the apparent age of 0 62 Myr for sample 73–650 from the overlying Adamstown Volcanics The Christians Cave formation can thus be interpreted as contemporaneous with an early phase of the Adamstown Volcanics Sample 73–640 gives a similar age (0 63 ± 0 01 Myr) and was collected from the south coast The radiometric age supports its inclusion in the Adamstown Volcanics as tentatively suggested by Carter[1] The two remaining samples from the Adamstown Volcanics have measured ages that are significantly younger (0 53 Myr and 0 45 Myr) Sample 73–654 is from a biotite mugearite flow, from which samples were taken by early Polynesians to manufacture artefacts The Pulawana Volcanics, which overlie stratigraphically the Tedside Volcanics, near to the west coast, give concordant K–Ar ages of 0 62–0 63 Myr on two samples A trachyte dyke (73–648) cutting the Pulawana Volcanics near the western tip of the island gives a slightly older age of 0 67 ± 0 01 Myr These ages are indistinguishable from the older ages found for the Adamstown Volcanics, which supports the suggestion[1] that the two formations are in part equivalent

We interpret these data as indicating that the Tedside volcanics, regarded as a relic of a dome building phase, were erupted between about 0 8 Myr and 0 9 Myr ago Following a hiatus of about 0 2 Myr, during which time caldera subsidence occurred, the Christians Cave Formation, the Adamstown Volcanics (which filled the caldera), and the extracaldera flows of the Pulawana Volcanics, were erupted about 0 62 Myr ago, with volcanism continuing until at least about 0 45 Myr ago The volcano was therefore active for a period of 0 5 Myr during the younger part of the Pleistocene

The Adamstown Volcanics and Christians Cave Formation are normally magnetised[1], which is consistent with their geochronological placing in the Brunhes normal magnetic epoch This study suggests that the Pulawana Volcanics also should have normal polarity Field compass texts indicated that the Tedside Volcanics may be normally magnetised This suggests that Tedside Volcanics were erupted during the short Jaramillo normal polarity event (about 0 90 Myr BP) during the Matuyama reversed polarity epoch

Migration of volcanism

Potassium–argon age determinations indicate that the exposed lavas on Pitcairn Island were erupted between 0 45 Myr and 0 93 Myr ago, and the island is therefore a relatively young feature in the south-eastern Pacific Ocean Its young age is incompatible with the estimated age of the surrounding sea floor and we conclude that Pitcairn Island could not have been formed at the East Pacific Ridge and then drifted northwest Its relationship to other areas of recent volcanism within the Pacific Ocean basin—the islands at the south-eastern end of the conspicuously linear and almost parallel Pacific island chains (the Hawaiian Islands, the Marquesas Islands, the Austral Islands and the Society Islands, Fig 1)—is not immediately clear, because of the absence of a neighbouring chain of volcanic islands The possibility that a nearby seamount chain exists, cannot be dismissed until more detailed bathymetry has been undertaken Pitcairn Island lies, however, very near to the south-eastern extension of the Duke of Gloucester Islands–Gambier Islands lineament, which is also approximately parallel to the Pacific island chains Potassium-argon age determinations on volcanic samples from the Gambier Islands[10] suggest that the tholeiitic and alkali olivine basalt lavas which form the islands of Mangareva, Aukena and Makapu, cooled between 5 2 Myr and 7 2 Myr ago Winterer[11] has reported an age of 8 Myr (D Krummenacher, personal communication) for basalt from a drill hole at Mururoa Atoll at the north-western end of the Gambier Islands Thus, there is the suggestion of a NW–SE migration of volcanism, which supports other geochronological studies of linear Pacific island chains[12–15]

If Pitcairn Island is a more recent manifestation of the source which produced volcanism in the Gambier Islands (and earlier, presumably, in the Duke of Gloucester Islands), then that source has migrated relative to the Pacific plate at a rate of approximately 11 cm yr^{-1} This value falls within previously determined migration rates of Pacific island chains, which range from 15 cm yr^{-1} in the Hawaiian Islands[15], through 10 cm yr^{-1} in the Marquesas Islands[12], to 9 cm yr^{-1} in the Austral Islands[13,14]

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Methylation of 16S RNA during ribosome assembly *in vitro*

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Methyl deficient 16S RNA from kasugamycin resistant Escherichia coli *has been used to study methylation of RNA during ribosome assembly* in vitro *Methylation occurs at an intermediate stage and is inhibited at a late stage of assembly 30S ribosomal proteins required for methylation, and inhibition, have been identified*

RIBOSOMAL RNA is synthesised *in vivo* as a large precursor which is reduced in length and methylated during ribosome assembly[1–6] Sequential addition of ribosomal proteins and conformational changes occur during maturation, resulting in the formation of mature ribosomes[6] Nomura and coworkers have studied the *in vitro* assembly of ribosomes using fully methylated mature 16S RNA Little is known, however, about the role of ribosomal proteins in the processing of precursor 16S RNA[7] Recent experiments which demonstrated that 16S RNA from kasugamycin-resistant *E coli* is undermethylated, provide an approach to an understanding of methylation and the sequence of events that occur during assembly of 30S ribosomes

Mutations at the *ksgA* locus in *E coli* result in kasugamycin resistance due to the lack of a methylase activity[8–10] Helser *et al* demonstrated that resistance or sensitivity to the antibiotic kasugamycin is determined by the nature of 16S RNA, 16S RNA from the resistant strain differs from the sensitive strain in that it lacks dimethylation of two adjacent adenines in a sequence of $m_2^6A\ m_2^6ACCUG$ near the 3′-OH end of the molecule[8] Helser *et al* have also demonstrated a direct cause and effect relationship between adenine dimethylation and sensitivity to the drug by first methylating the resistant 16S RNA *in vitro* followed by reconstitution of 30S subunits with this RNA[9]

Isolated 'kasugamycin-resistant' 16S RNA could not be methylated *in vitro* in these studies, but the ribonucleoprotein particles prepared by CsCl density gradient centrifugation (CsCl cores) were active substrates This is consistent with the notion that methylation occurs during ribosome assembly We report here our studies on methylation of 16S RNA from a kasugamycin-resistant strain during 30S ribosome assembly *in vitro*

Purification and properties of methylase

The methylase was purified from *E coli* A19 (RNase I^-, General Biochemicals) Cells were disrupted in a French press in TMAI buffer (10mM Tris-HCl, *p*H 7 6, 30 mM NH_4Cl, 10 mM $MgCl_2$ and 6 mM 2-mercaptoethanol), the ribosomes separated by centrifugation and washed overnight in IF buffer (20 mM Tris-HCl, *p*H 7 8, 30 mM $MgCl_2$, 1 M NH_4Cl, 6 mM 2-mercaptoethanol) The salt-washed ribosomes were pelleted and the high speed supernatant was used for purification of the enzyme The supernatant was dialysed first against TMAI buffer overnight and then against phosphate buffer (50 mM NaH_2PO_4, *p*H 5 8, 1 5 mM 2-mercaptoethanol) containing 10% glycerol and adsorbed on to a phosphocellulose column equilibrated with the same buffer The column was washed with phosphate buffer containing 0 25 M NaCl and the enzyme was eluted with a linear salt and *p*H gradient (0 25–0 65 M NaCl), *p*H 5 8–6 5) The methylating activity eluted as a major peak near 0 55 M NaCl with a small shoulder The major peak fractions were pooled and dialysed against TMAI buffer (containing 10% glycerol) and used as a source of methylase The conditions for assay of column fractions for methylase activity were as reported previously[8] The methylase obtained by this procedure is 60–80-fold purified over crude extracts and remains stable when frozen at –70° C in TMAI buffer containing 10% glycerol Further purification of the enzyme resulted in loss of activity, hence the phosphocellulose enzyme was used in all experiments 30S ribosomes, CsCl core particles and 16S RNA were prepared from kasugamycin-resistant *E coli* strain TPR 201 according to methods described by Traub *et al*[9,11]

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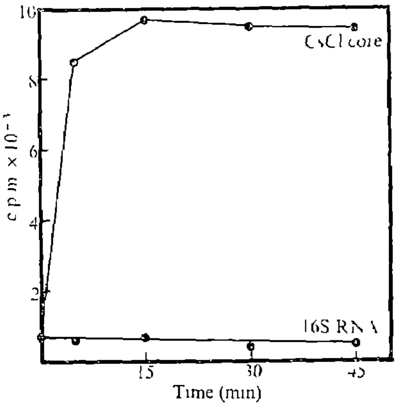

Fig 1 Time course of methylation of CsCl core particles obtained from kasugamycin-resistant 30S ribosomal subunits 0 25 A_{260} units of CsCl core particles (16 6 pmol) or 16S RNA (20 pmol) were incubated in 30 µl of reconstitution buffer (30 mM Tris-HCl, *p*H 7 4 20 mM $MgCl_2$, 6 mM 2-mercaptoethanol and 330 mM KCl) for 30 min at 40° C These conditions are referred to as 'reconstitution conditions'[12] The tubes were then chilled on ice and 5 µl of partially purified methylase (~1 µg) and 1 µl of ^3H-methyl-labelled S-adenosyl L-methionine specific activity 500 µCi µmol^{-1}) were added The reaction mixtures were diluted to 100 µl with methylase buffer (50 mM Tris-HCl, *p*H 7 8, 45 mM NH_4Cl, 4 mM $Mg(OAc)_2$) and assayed for the indicated times at 37° C The reaction was stopped by adding 1 ml of 10% trichloroacetic acid (TCA) and 0 2 ml of 0 2 mg ml^{-1} bovine serum albumin The precipitates were collected, washed with 5% TCA and counted Under these conditions, 10,000 c p m correspond to 68 pmol of methyl groups incorporated (About 5–10% background radioactivity was usually observed in RNase treated controls These background counts were not subtracted from the data presented The RNase sensitive maximum methylation corresponds to 3 3 methyl groups per core particle)

Figure 1 shows the kinetics of methylation with kasugamycin-resistant core particles as a substrate, 16S RNA is not a substrate for methylation The extent of methylation of core particles is comparable with that reported by Helser *et al*[8] Subsequently, 30S ribosomes were reconstituted from CsCl core particles and split proteins (SP30) under the conditions used for total reconstitution of 30S ribosomes[11,12] The resulting particles were assayed for methylation without isolation As Fig 2 shows, addition of split proteins to produce 30S ribosomes resulted in an inhibition of methylation

These results indicate that 16S RNA is not a substrate for methylation and that binding of some ribosomal proteins is essential for methylation to occur Completion of assembly of 30S ribosomes must result in a structure in which the AACCUG sequence is in an unreactive state for methylation

To determine which proteins are involved in conferring substrate specificity to 16S RNA, we studied the methylation of 16S RNA in ribonucleoprotein complexes with different protein compositions Purified 30S proteins were mixed in three groups as described in the legend to Table 1 These three groups included all the proteins present in the core particles as well as S5 (according to the accepted nomenclature of Wittmann *et al*[13] S5 was included in the mixture to ensure efficient binding of S21 (Fig 4)[14]

16S RNA was incubated with groups of proteins under reconstitution conditions and the mixture was assayed for methylation of the resulting ribonucleoprotein complexes as described previously Table 1 shows that all three groups of proteins (*A*, *B*, and *C*) were required for methylation of 16S RNA (Table 1*a*, line 8) Omission of any group gave a non-methylatable complex (Table 1*a*) In addition, the results indicate that the formation of an RNA–protein complex which can be methylated is temperature-dependent Incubation at 0° C and assay for methylation at 37° C resulted in reduced methylation (Table 1*b*) The observed low level of methylation was presumably due to some reconstitution during the assay at 37° C

Several single-component omission experiments were done to determine whether all proteins in each group were essential for methylation (Table 2) Omission of S13 and S20 from group *A* did not affect methylation but the omission of any one of the proteins S4, S8, or S15 caused a reduction in methylation (Table 2*a*) Addition of S4, S8, and S15 in combinations of two to groups *B* and *C* did not bring about methylation (Table 2*d*) Thus, three of the proteins (S4, S8 and S15) in group *A* are needed for methylation (Table 2*d*, line 4) Single-omission experiments (Table 2*b*) suggest that only S16 of group *B* is required for methylation In the presence of groups *A* and *C*, however, addition of S16 only was not sufficient for maximum stimu-

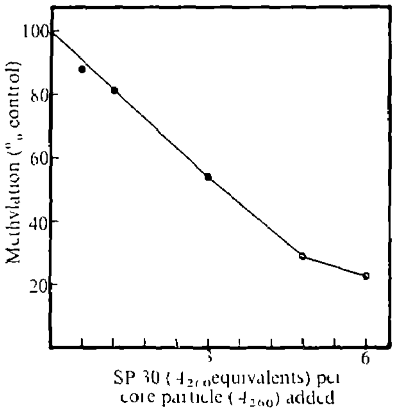

Fig 2 Inhibition of methylation after reconstitution of 30S ribosomes from core particles and split proteins CsCl core particles and split proteins (SP30) were prepared as described by Traub *et al*[11] The split proteins (SP30) were dialysed against TRI buffer (30 mM Tris-HCl, *p*H 7 4, 20 mM $MgCl_2$, 6 mM 2-mercaptoethanol and 1 M KCl) 30S ribosomes were reconstituted under 'reconstitution conditions' as described in Fig 1 and the resulting particles were methylated essentially as described in the legend to Fig 1 One A_{260} equivalent of SP30 corresponds to the split proteins obtained from 1 A_{260} unit of 30S ribosomes[11] 100% methylation corresponds to 3 3 methyl groups per core particle

Table 1 Reconstitution of core structure for methylation

			c.p.m.	%
(a)	(1)	16S	1,047	15
	(2)	16S + Gp *A*	1,406	21
	(3)	16S + Gp *B*	1,046	15
	(4)	16S + Gp *C*	918	14
	(5)	16S + Gp *A* + Gp *B*	1,916	29
	(6)	16S + Gp *A* + Gp *C*	2,073	31
	(7)	16S + Gp *B* + Gp *C*	1,340	20
	(8)	16S + Gp *A* + Gp *B* + Gp *C*	6,641	100
	(9)	Core particle	8,100	122
(b)		16S + Gp *A* + Gp *B* + Gp *C* $\xrightarrow[30']{0°}$	2,563	39

Proteins in various groups are *A*, S4, S8, S20, S15 and S13, *B*, S7, S16, S17, S19, and S5, *C*, S6, S18, S11, S21 and S12 30S ribosomal proteins were purified from *E. coli* Q13 as described by Held *et al.*[12] Purified ribosomal proteins were dialysed against reconstitution buffer containing 0.5 M KCl. Particles of indicated protein composition were reconstituted by incubating 0.25 A_{260} units of kasugamycin-resistant 16S RNA with a 2 molar excess of pure proteins. Reconstitution and methylation assays were as described previously. In the experiment in (*b*), 16S RNA was incubated in the reconstitution buffer with the indicated proteins at 0° C for 30 min and assayed for methylation at 37° C. 100% methylation (6,641 c.p.m.) corresponds to 2.3 methyl groups per 16S RNA. The extent of methylation (100% values) varied from 2.0 to 3.5 methyl groups per 16S RNA in different experiments. This variation may reflect differences between batches of 16S RNA and proteins used for reconstitution as well as variability of the components of the methylase reaction.

lation of methylation. Both S16 and S17 were required (data not shown, see also below). Thus S16 and S17 are the only proteins needed from group *B*, S5, S7, and S19 are dispensable.

Single omission of proteins in group C indicate that S6, S11, and S18 are needed for methylation of 16S RNA together with the proteins in groups *A* and *B* (Table 2*c*).

Table 2 Effect of omission of single proteins on the methylation

			% Methylation
(a)	(1)	16S	3
	(2)	Core particle	124
	(3)	16S + Gp *B* + Gp *C* + Σ Gp *A*ᵢ	100
	(4)	− S15	57
	(5)	− S13	100
	(6)	− S20	92
	(7)	− S8	16
	(8)	− S4	31
(b)	(1)	16S + Gp *A* + Gp *C* + Σ Gp *B*ᵢ	100
	(2)	− S5	101
	(3)	− S7	104
	(4)	− S19	111
	(5)	− S16	36
	(6)	− S17	101
(c)	(1)	16S + Gp *A* + Gp *B* + Σ Gp *C*ᵢ	100
	(2)	− S21	102
	(3)	− S6	79
	(4)	− S18	65
	(5)	− S12	110
	(6)	− S11	63
(d)		Effect of addition of single proteins to protein mixtures on methylation	
	(1)	16S + Gp *B* + Gp *C* + S4 + S8	74
	(2)	+ S4 + S15	10
	(3)	+ S8 + S15	15
	(4)	+ S4 + S8 + S15	108

Particles were reconstituted as described previously. The protein composition of groups *A*, *B* and *C* is given in Table 1. In single-omission experiments shown in (*a*), (*b*) and (*c*) the indicated purified proteins were omitted from the reaction mixtures. In (*d*) the indicated proteins were added to mixtures of group *B* and group *C* proteins. 100% methylation corresponds to 2.7 (7,956 c.p.m.), 2.0 (5,976 c.p.m.) and 2.7 (6,596 c.p.m.) methyl groups per 16S RNA for (*a*), (*b*) and (*c*), respectively.

Addition of S6, S11 and S18 individually, or in combinations of two, to groups *A* and *B* does not lead to significant methylation (data not presented here). Thus three proteins (S6, S11, and S18) of group *C* are required for methylation.

These results indicate that at least eight of the 14 proteins present in the core particle (S4, S8, S15, S16, S17, S18, S11 and S6) are necessary for methylation of 16S RNA. Table 3 shows that a methylatable substrate can be formed by addition of this set of eight proteins to 16S RNA. The extent of methylation is almost the same as that observed with particles reconstituted with a mixture of all the core proteins (Table 3, line 5). Thus, the interaction of 16S RNA with eight proteins is needed to form a minimum structure required for methylation. Omission of any one of the proteins in the minimum structure leads to reduced methylation. Omission of S4, S8, S16 or S17 leads to a more drastic reduction in methylation than other proteins.

Inhibition of methylation

As pointed out earlier, Fig. 2 shows that reconstitution of 30S ribosomes from CsCl core particles and split proteins results in inhibition of methylation. To determine whether one or more of the 'split' proteins may be involved in the inhibition, we performed several single component omission experiments in which each one of the 'split' proteins (S1, S2, S3, S5, S9, S10 and S14) was omitted from reconstitution mixtures[7,11].

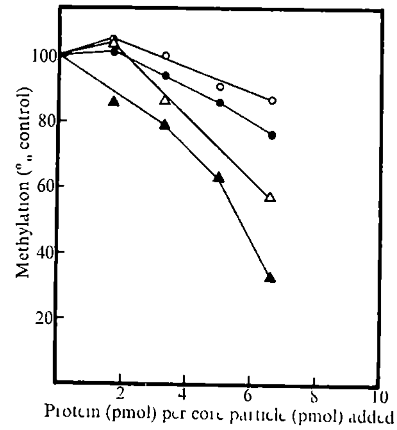

Fig. 3 Effect of single protein omission on methylation of reconstituted 30S ribosomes. *E. coli* A19 salt-washed ribosomes prepared as described in the text were dissolved in TMAI and sedimented through 30% sucrose in TMAI. The ribosomal subunits were separated by zonal ultracentrifugation in a Ti 15 rotor (5–30% sucrose gradient in 10 mM Tris-HCl, *p*H 7.4, containing 0.3 mM $MgCl_2$, 30 mM NH_4Cl and 6 mM 2-mercaptoethanol). 30S ribosomal proteins were extracted and purified essentially as described by Hardy *et al.*[24] The purified split proteins were dialysed against reconstitution buffer containing 0.5 M KCl before reconstitution. Ribosomes were reconstituted from CsCl core particles and purified split proteins. Proteins omitted from reconstitution mixtures are indicated in the figure. Reconstitution and assay conditions are as previously described. 100% methylation corresponds to 3.3 methyl groups per core particle. ○, minus S3; ●, minus S14(S9); △, minus S10; ▲, minus S2 (S1, S5).

Figure 3 shows that omission of S1, S2 or S5 did not affect inhibition of methylation by the rest of the split proteins. Omission of S3, S9 or S14 resulted in a considerable reduction in the inhibition of methylation and omission of S10 partially reduced the inhibition. Addition of any one of the purified split proteins to the core particles does not inhibit methylation (data not shown here) It should be noted that the extent of inhibition observed in experiments with purified split proteins was somewhat less than with unpurified split proteins (data in Fig 2)

We next asked if the inhibitory proteins S3, S9, S10 and S14 are sufficient to inhibit methylation of the minimum methylatable structure established above (Table 3) Three proteins (S7, S19 and S5), not required for methylation, were also added since they are necessary for the binding of proteins S3, S9, S10 and S14 (Fig 4)[14] Methylation was not inhibited when the inhibitory protein were added together with S7, S19 and S5 (Table 4, line 3) The presence of the additional proteins may have stabilised the minimum structure and thus stimulated the reaction

Table 3 Establishment of minimum core structure for methylation

		% Methylation
(1)	16S	9
(2)	16S + Gp *A* + Gp *B* + Gp *C*	100
(3)	16S + Gp I	15
(4)	16S + Gp II	11
(5)	16S + Gp I + Gp II	89
(6)	– S4	13
(7)	– S8	14
(8)	– S16	17
(9)	– S17	19
(10)	– S15	33
(11)	– S18	42
(12)	– S11	42
(13)	– S6	44

Proteins present in group I are S4, S8, S16 and S17, and Group II, S15, S18, S11 and S6 100% methylation corresponds to 2 2 (6,576 c p m) methyl groups per 16S RNA

Thus, in the experiment shown in Table 4 (line 3) methylation was not inhibited by the inhibitory proteins even though all the proteins present in core particles were present with the exception of S20, S21, S13 and S12 Addition of each one of these four proteins to the mixtures did not result in inhibition (Table 4, lines 4–7), but addition of S20 and S21 together resulted in a significant inhibition of methylation (Table 4, line 9) S12 slightly enhanced the inhibition observed with S20 and S21 (Table 4, line 13)

Our results can be summarised as follows (1) 16S RNA is not methylated but CsCl core particles are active substrates (2) Interaction of eight proteins (S4, S6, S8, S11, S15, S16, S17 and S18) with 16S RNA results in the formation of a minimum structure which can be methylated (3) Addition of split proteins to core particles leads to an inhibition of methylation Proteins S3, S9, S10 and S14 are the split proteins responsible for inhibition S1, S2 and S5 are not involved in inhibition (4) In addition to the inhibitory split proteins, the inhibition of methylation of 16S RNA is observed only in the presence of most of the core proteins, several of which are not essential for methylation (that is S7, S19, S20 and S21)

Methylation and assembly of 30S ribosomes

In the experiments reported here, the 30S ribosomal proteins required for *in vitro* methylation of 16S RNA, as well as those involved in inhibition of this reaction, have been identified Table 3 shows that eight 30S protein (S4, S8, S15, S16, S17, S18, S11 and S6) are required for methylation of the AACCUG sequence in 16S RNA *in vitro*

Six 30S proteins (S4, S7, S8, S15, S20 and S17) have been shown to interact with 16S RNA directly in the absence of other proteins in a site-specific manner[14–19] Four of these proteins, S4, S8, S15 and S17, are necessary for *in vitro* methylation of 16S RNA Zimmermann *et al* reported that S4, S8 and S15 (as well as S20 and possibly S13) interact specifically with an RNA fragment toward the 5′ half of the 16S RNA[20] The m$_2^6$A m$_2^6$A CCUG sequence is about 25 nucleotides from the 3′–OH end of 16S RNA[21] Thus, several proteins (S4, S8 and S15) which are known to bind some distance from the site of methylation are nonetheless necessary to establish an RNA–protein structure which can be methylated This suggests that these proteins are required for a structural change which affects the substrate specificity of the 3′–OH end of the RNA for methylation It is interesting that S7, which is the only protein known to bind directly near the 3′–OH end of 16S RNA[20], and hence close to the methylation site, is not required for methylation (data described in Table 3)

A comparison of the proteins required for methylation of 16S RNA and the assembly map (Fig 4)[14 16] indicates these proteins are strongly interrelated and occupy an early part of the map In addition to S4, S8, S16, and S17, the group of interrelated proteins shown on the left side of the assembly map (S15, S18, S6 and S11) are required for methylation while all of the proteins on the right side of the map whose binding strongly depends on the prior binding of S7, are not required This latter group of proteins, however, inhibits methylation (that is S9, S14, S10 and S3) It is probable that *in vivo* the methylating enzyme acts on intermediate particles which contain the essential eight proteins and perhaps other proteins, but lack some or all of the inhibitory proteins

Analysis of the protein composition of ribosomal precursor particles is consistent with the early incorporation of the eight proteins required for methylation during *in vivo* assembly 21S particles accumulated at low temperatures by a cold-sensitive mutant of *E coli* contain all the proteins found to be required for methylation with the exception of S11[22] In addition to the set of seven proteins, these particles also contain proteins which are not required for methylation (S7, S19, S20, S13 and possibly S9) It should be pointed out that these *in vivo* particles contain precursor 16S RNA which is not methylated Five of the eight proteins required for methylation (S4, S8, S15, S16 and S17) are also present in the *in vivo* 22S particles studied by Nierhaus *et al*[23]

Table 4 Inhibition of methylation of minimum core structure

		% Methylation
(1)	16S	19
(2)	16S + Gp I + Gp II	100
(3)	16S + Gp I + Gp II + Gp III	125
(4)	+ S20	113
(5)	+ S12	129
(6)	+ S21	116
(7)	+ S13	132
(8)	+ S20 + S12	100
(9)	+ S20 + S21	65
(10)	+ S20 + S13	104
(11)	+ S20 + S12 + S13	97
(12)	+ S20 + S13 + S21	68
(13)	+ S20 + S12 + S21	54
(14)	+ S12 + S13	120
(15)	+ S12 + S21	99

The protein composition of groups I and II are the same as that given in Table 3 Proteins present in group III are S7, S19, S3, S5, S9, S10 and S14 100% methylation corresponds to 3 5 (10,287 c p m) methyl groups per 16S RNA

Our results indicate that the eight proteins required for RNA methylation *in vitro* should be present during an intermediate step of ribosome assembly *in vivo* (that is, before the methylation of the m$_2^6$A m$_2^6$A sequence) Such an intermediate may contain some proteins which are not required for the methylation reaction we studied In addition, some of the proteins found to be required for methylation of the m$_2^6$A m$_2^6$A sequence may also be necessary for methylation of other RNA sequences during ribosome assembly It is also possible that other 30S proteins are specifically required for some of the methylations other than the dimethylation of adenines It is probable that the methylation of both adenines in the AACCUG sequence occurs even when partial methylation is observed[9] It is possible, however, that methylation is a sequential process in which the degree and type of methylation is determined by the composition and structure of the intermediate ribonucleoprotein particle

Methylation of the AACCUG sequence in 16S RNA is inhibited by 30S proteins which are incorporated during the final stages of 30S ribosome assembly (Table 4 and Fig 4) Four split proteins S3, S9, S10 and S14 are responsible for the inhibition of methylation of core particles (Fig 3 In addition to the inhibitory split proteins, we have found that several core proteins also contribute to inhibition of methylation (data in Table 4)

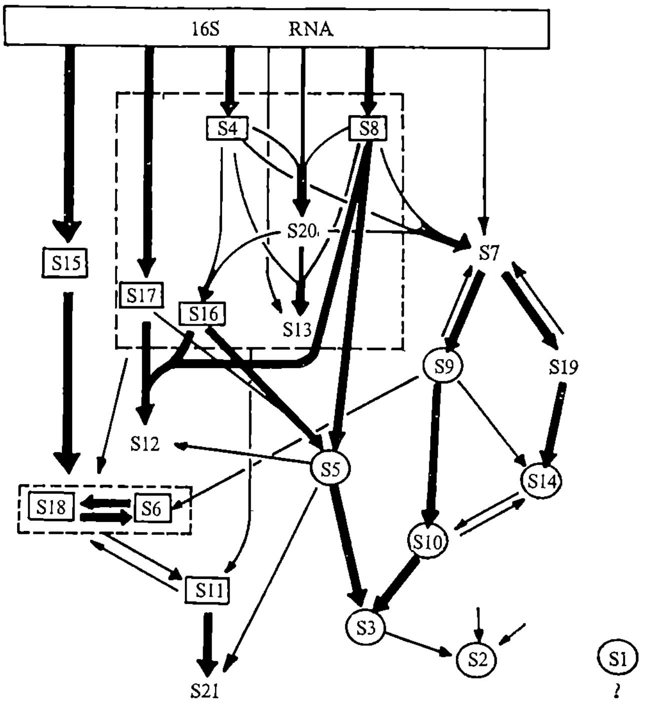

Fig 4 The 30S ribosome assembly map and the proteins required for methylation of 16S RNA The assembly map is from the work of Mizushima and Nomura[14] and Held *et al*[16] An arrow from 16S RNA to a protein indicates that the protein binds directly to 16S RNA in the absence of other proteins Arrows between proteins indicate the facilitating effect of one protein on the binding of another A thick arrow indicates a strong facilitating effect and a thin arrow, a weak facilitating effect The 30S proteins required for methylation of *ksg* 16S RNA are boxed CsCl split proteins are circled

In earlier experiments, occasional methylation of 30S ribosome preparations were observed This was thought to be due to partial unfolding of particles in such preparations[9] Subsequently, it was observed that methylation of 30S particles could be inhibited by the addition of split proteins, but with about one-quarter the amount needed for core particles (our unpublished observation) Thus we believe that the occasional methylation of 30S particles is due to a deficiency of one or more of the inhibitory proteins in the isolated 30S ribosomes This interpretation is consistent with the observation that the inhibitory proteins S3, S10 and S14 are present in amounts of less than one copy per isolated 30S particle[15]

The inhibition of methylation is a complex phenomenon Some of the proteins involved in the inhibition of methylation might make the site of methylation inaccessible to the methylase as a result of blocking the sequence to be methylated, while other proteins may facilitate the binding of inhibitory proteins In addition, specific changes in RNA conformation near the 3′-OH end might also be responsible for the observed inhibition

By using an approach similar to that used here, it should be possible to study the various steps in the maturation of precursor RNA and methylation of sequences other than the m$_2^6$A m$_2^6$A sequence Such studies would give information regarding the role of 30S proteins during maturation and assembly of the ribosome

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Size and location of the transforming region in human adenovirus type 5 DNA

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Fragments of human adenovirus type 5 DNA as small as one million daltons can transform cells in vitro. The DNA segment which induces transformation is located between 1 and 6% from the left end of the Ad 5 DNA molecule.

HUMAN adenoviruses contain linear double stranded DNA of molecular weight 20 × 10^6 to 25 × 10^6 (refs 1, 2). They can be classified into three subgroups A, B, and C which vary in their degree of oncogenicity[3]. Viruses of subgroup C (to which adeno 5 belongs) are nononcogenic in that they do not give rise to tumours after injection into newborn hamsters[4], they can, however, transform cells *in vitro*[5,6] and in certain conditions the transformed cells can induce tumours in animals[7,8].

We have recently shown that Ad 5 DNA is infectious for human KB cells[9] and that it can transform rat cells *in vitro*[10]. Although DNA from simian adenovirus 7 had previously been reported to be oncogenic in newborn hamsters[11] our observations provided the first demonstration of transformation *in vitro* by adenovirus DNA. The development of an *in vitro* assay[10] afforded the opportunity for quantitative studies of transformation by adenovirus DNA.

While examining the effects of various treatments on the biological activity of Ad 5 DNA we observed that transforming activity was much more resistant to shearing than was infectivity. DNA preparations which were rendered noninfectious by shearing still retained undiminished transforming activity. We concluded that we might be observing transformation by DNA fragments. The studies presented here were undertaken to prove that Ad 5 DNA fragments of less than genome size have transforming activity, to determine the minimum size of fragments which retain the ability to transform cells, and to map the location of the transforming (T) segment within the viral DNA molecule.

The assays for biological activity were carried out using a modification of what we now call the calcium technique[9]. This method is based on the formation of a coprecipitate of DNA and calcium phosphate (formed by adding $CaCl_2$ to solutions of DNA in phosphate-containing buffer) which on addition to cell cultures becomes associated with the cells. DNA uptake then takes place at 37° C by a process which again requires calcium ions. The most important modification which we have introduced in the technique is the addition of a carrier DNA to all assays. This was shown to improve the efficiency of the assay[9] and has the important advantage of producing a linear dose response (ref 12 and Fig 1), thus simplifying quantitative calculations of specific activities.

Transformation by sheared Ad 5 DNA

Ad 5 DNA which was sheared to fragments having a sedimentation coefficient ($S_{20,w}$) of 22S (molecular weight 8 × 10^6) had the same transforming activity as unsheared DNA (Fig 1) yet infectivity of the sheared DNA could not be detected when

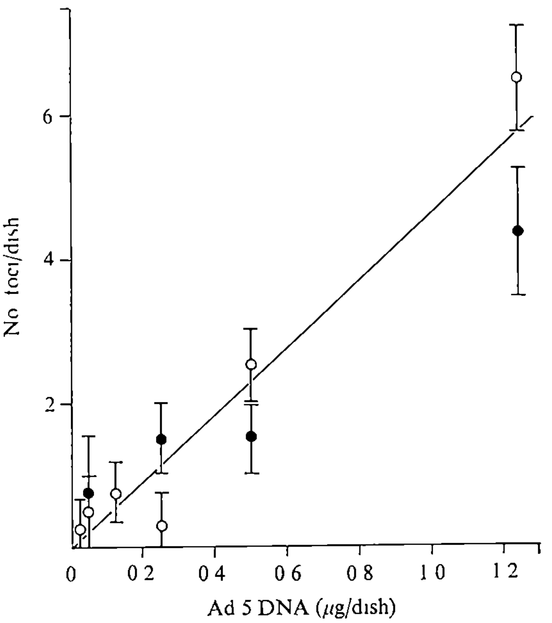

Fig 1 Dose response for transformation of primary rat kidney cells by intact (○) and sheared (●) Ad 5 DNA. The DNA was extracted from purified Ad 5 as described previously[1]. Shearing was carried out by passing 200 μg ml^{-1} solutions of Ad 5 DNA in 0.1×SSC (15 mM NaCl, 1.5 mM sodium citrate) 10 times through a needle (0.72 mm inside diameter×30 mm long) using maximum hand pressure on a syringe with a 15 mm diameter plunger. All sedimentation coefficients were determined by band sedimentation in 1 M NaCl at 20° C at an initial DNA concentration of approximately 25 μg ml^{-1} in an analytical ultracentrifuge equipped with ultraviolet optics and a photoelectric scanner. Coefficients have been corrected for viscosity but not for concentration dependence. Intact Ad 5 DNA sedimented at 30S and DNA sheared as described above sedimented at 22S. The assays for transforming activity were based on previous studies on infectivity[9] and transforming activity[10] of Ad 5 DNA and were carried out on subconfluent cultures of primary rat kidney cells prepared by trypsin dispersion of kidneys from week old Wistar rats. Viral DNA was diluted into HEPES buffered saline (HeBS: 8.0 g l^{-1} NaCl, 0.37 g l^{-1} KCl, 0.125 g l^{-1} $Na_2HPO_4 \cdot 2H_2O$, 1.0 g l^{-1} dextrose, 5.0 g l^{-1} N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid, final *p*H 7.05) containing salmon sperm DNA at 10 μg ml^{-1}. $CaCl_2$ was added to 125 mM and the mixture was incubated at room temperature for 10–20 min during which time a precipitate formed. Aliquots (0.5 ml per dish) of the resulting suspension (consisting of a co-precipitate of DNA and calcium phosphate) were then added to rat kidney cells growing in 60 mm plastic Petri dishes containing 5 ml Eagle's minimum essential medium supplemented with 10% calf serum (MEM+10% CS) and the dishes were placed in a 37° C, 5% CO_2 incubator. After 3–4 h at 37° C the medium was removed and replaced with fresh MEM +10% CS and the incubation at 37° C continued. After 3–4 d the medium was replaced with low calcium medium[6] (MEM for suspension cultures+0.1 mM $CaCl_2$+5% CS) and this was refreshed at 3–4 d intervals. At 3 weeks, by which time few normal cells remained attached to the dishes, the cultures were fixed, stained with Giemsa, and transformed colonies were counted. Each point represents the average of 4 dishes and the error bars represent ±1 s.d.

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assayed in conditions which resulted in 20–30 PFU μg^{-1} for intact DNA Furthermore, permissive cells (hamster and human) which could not be transformed by intact DNA (due to the resulting productive infection which spread to all the cells in the culture) could be transformed by noninfectious sheared DNA These observations showed that transformation did not require infectious DNA molecules and suggested that DNA fragments smaller than a viral genome had the ability to transform They did not, however, eliminate the possibility that transforming activity was associated with a shear-resistant (for example, circular) molecular form of Ad 5 DNA To rule out this possibility intact and sheared DNA preparations were fractionated through sucrose gradients and fractions were assayed for transforming activity

Activity should sediment at the same rate before and after shearing if it were associated with a shear resistant form of Ad 5 DNA The results shown in Fig 2 clearly demonstrate that this was not the case Transforming activity of unsheared preparations sedimented with the same velocity as intact DNA and shearing reduced the sedimentation velocity and transforming activity of the DNA to the same extent (Fig 2*a* and *b*) The results shown in Fig 2*c* indicate that even DNA fragments smaller than 15S (3 × 10^6 daltons) could transform with an efficiency as high as that of intact DNA Thus transforming activity appeared to be retained even after breaking the DNA to fragments as small as 1/8 of the viral genome It was now of considerable interest to determine more precisely the degree to which Ad 5 DNA could be fragmented before all transforming activity was lost

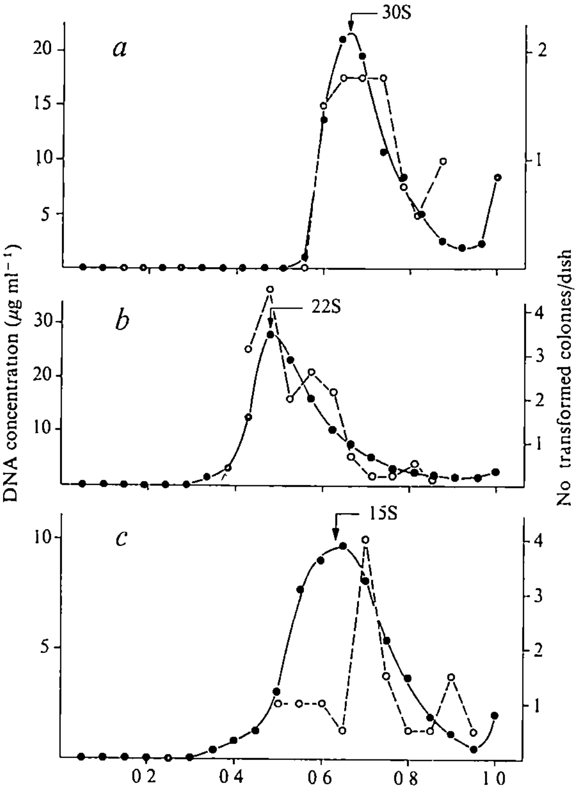

Fig 2 Transformation by intact and sheared Ad 5 DNA fractionated by velocity sedimentation Aliquots of ^{3}H-Ad 5 DNA (2,000 c p m μg^{-1}) were layered on to 5–22% exponential sucrose gradients containing 1 M NaCl, 0 01 M Tris, *p*H 8.1, and 0 5 mM disodium ethylenediaminetetraacetate which had been prepared by pumping sucrose solutions from a 70 ml mixing chamber connected to a reservoir containing 50% sucrose Gradients were centrifuged at 4° C for 22 h (*a* and *b*) or 34 h (*c*) at 25,000 r p m in a SW 27 rotor and fractionated from the top DNA concentrations were determined by counting 25 μl and transforming activity was measured as described in the legend to Fig 1 (50 μl DNA was assayed per dish) Recovery of radioactivity was 60–70% The sedimentation coefficients given in the figure were determined by band sedimentation in the analytical ultracentrifuge before the DNA was fractionated through sucrose gradients *a*, Intact DNA Transforming activity was measured in two separate experiments, two dishes/fraction The points therefore represent the mean of four dishes *b*, DNA sheared to 22S according to the legend to Fig 1 Each point represents the mean of two separate assays on two dishes each *c*, DNA sheared by passage 20 times through a 0 42 mm × 22 mm long needle using maximum hand pressure on a syringe with a 9 mm plunger Fractions were assayed once for transformation, 2 dishes per assay ●, DNA concentration, ○, Transforming activity

Minimum size of transforming fragments

If transformation by Ad 5 DNA required the integrity of some segment (the T segment) of length L then transforming activity of randomly fragmented DNA should decrease as the fragment size approached L and should be zero for fragments less than or equal to L in molecular weight To obtain an estimate of L, Ad 5 DNA was extensively fragmented by shearing or sonication (resulting in modal sedimentation coefficients of 11 5S and 12S, respectively), fractionated by velocity sedimentation, and assayed for transforming activity

The results, plotted in Fig 3*a* and *b*, showed that for both sheared and sonicated preparations, transforming activity was found only in fractions containing DNA fragments sedimenting faster than 10S To ensure that the molecular weight of the DNA fragments had not changed between the time they were prepared and the time they were assayed for transforming activity, and to determine as accurately as possible the molecular weight of DNA fragments in the region where transforming activity approached zero, peak fractions from the gradients in Fig 3*a* and *b* were concentrated after transforming activity had been measured and sedimentation coefficients were redetermined in the Model E ultracentrifuge The resulting values (shown in Fig 3*a* and *b*, and differing only slightly from the modal values obtained before fractionation) were then used to calibrate the gradients to determine molecular weights from the equations in the legend to Fig 3

The graph of specific transforming activity plotted versus molecular weight in Fig 3*c* shows that fragmentation of Ad 5 DNA, whether by shearing or sonication, began to reduce transforming activity when the fragment size fell below about 2 × 10^6 daltons and activity was completely abolished at slightly below 1 × 10^6 daltons If loss of activity at this point was due to the introduction of breaks into the T segment then the size of the T segment must be approximately 10^6 daltons But since there may be other explanations for absence of transforming activity below 10^6 daltons (for example, DNA fragments smaller than 10^6 daltons may be inherently lacking in any biological activity in our assay system) we can only conclude that the T segment is not greater than about 10^6 daltons in length

The possibility that transformation by small fragments required the interaction of two or more DNA segments can be ruled out since such a mechanism would result in a dose response of 2nd or higher order Yet we observed no significant departure from a first order dose response with decreasing fragment size It would appear, therefore, that only one small segment of the Ad 5 DNA molecule is required for transformation and that the molecular weight of this segment is not more than about 10^6 daltons

The properties of cells transformed by small DNA fragments are now under investigation We can only say that such transformed cells do not appear to differ morphologically from those transformed by intact DNA One line of rat kidney cells transformed by fragments of molecular weight 1 5 × 10^6 is now at the 27th passage in culture, several transformed hamster lines (transformed by DNA fragments of molecular weight about 8 × 10^6) have been maintained in culture for 5–23 passages and one transformed human cell line is now at passage 51 Furthermore, of five transformed hamster cell lines tested for tumorigenicity, all induced tumours when injected into newborn hamsters Thus DNA fragments seem to be capable of inducing not only stable, but also oncogenic transformation By indirect immunofluorescence using serum from tumour-bearing hamsters, we have demonstrated T antigen in all of several DNA-transformed cell lines tested, including one transformed by fragments of molecular weight 1 5 × 10^6 (The serum was obtained from hamsters bearing tumours induced by Ad 5 transformed cells and was pretested against transformed rat cells which had previously been shown positive for T antigen[10])

Location of the T segment

The finding that fragments of Ad 5 DNA had the ability to transform cells suggested the feasibility of mapping the T segment within the viral DNA molecule and of purifying this segment The first step which we took in this direction was to determine which half of the molecule contained transforming activity Since the DNA of Ad 5 (like that of Ad 2 (refs 14, 15)) has an asymmetric distribution of GC, it was possible to separate the two halves of Ad 5 DNA by equilibrium buoyant density centrifugation of half molecules in a Hg(II)-Cs$_2$SO$_4$ gradient according to the method of Nandi, Wang and Davidson[16] DNA was sheared, fractionated on sucrose gradients to purify the halves which were then separated in a Cs$_2$SO$_4$ gradient in the presence of Hg(II) Fractions corresponding to AT or GC-rich halves were pooled, dialysed against HeBS, and aliquots were analysed by buoyant density centrifugation in CsCl in a Model E analytical ultracentrifuge The results indicated that a single fractionation on a Hg(II)-Cs$_2$SO$_4$ gradient was sufficient to provide relatively pure preparations of AT-rich and GC-rich halves (the cross contamination was less than about 10%) When these preparations were assayed for transforming activity it was found that the AT-rich halves had less than 5% of the transforming activity observed for the GC-rich fractions (0 7 colonies per μg AT-rich

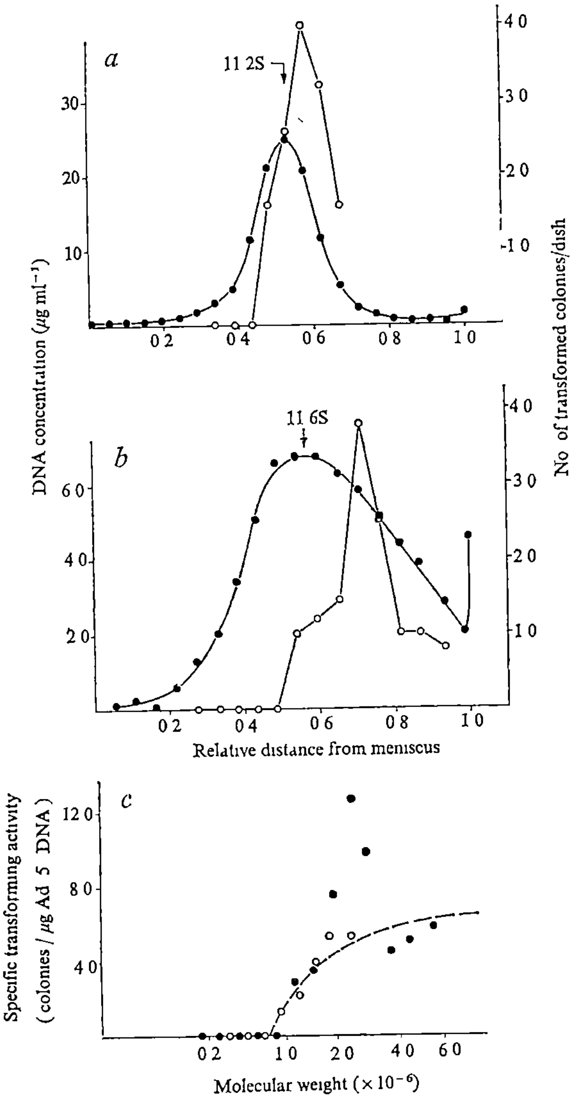

Fig 3 Transformation by extensively fragmented Ad 5 DNA *a*, DNA at 200 μg ml^{-1} was sheared for 3 min in a Sorvall omnimixer micro attachment by a stainless steel blade rotating at 40,000 r p m A sedimentation coefficient of 11 5S was determined for this DNA before it was fractionated on sucrose gradients DNA was sedimented by centrifugation at 23,000 r p m for 44 h on gradients prepared according to the legend to Fig 2 After fractionating and assaying aliquots for transforming activity (50 μl per dish) the peak fraction was concentrated and the sedimentation coefficient again measured to give the value shown in the figure *b*, DNA at 100 μg ml^{-1} was sonicated in a MSE sonicator for 15 s, at 20,000 c p s, amplitude 2 μm This resulted in a DNA preparation which sedimented in the analytical ultracentrifuge as a rather broad band having a modal sedimentation coefficient of approximately 12S (The coefficient shown in the figure was determined for the combined fractions 10 and 11 after assaying for transformation) ●, DNA concentration, ○, transforming activity, mean of 5 dishes per point *c*, Specific transforming activity of Ad 5 DNA fragments calculated from panels A (○) and B (●) as a function of molecular weight Molecular weights were calculated from the equations D=kS and S=0 0882 M$^{0\ 346}$ (ref 13) where D is distance sedimented and k was determined from the sedimentation coefficients shown in panels A and B

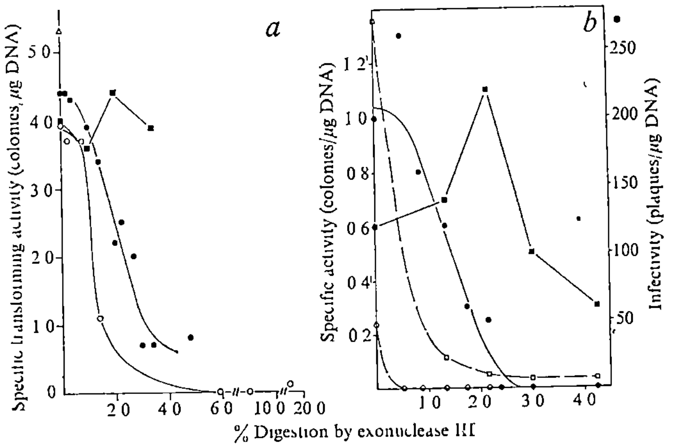

Fig 4 *a*, Transforming activity of Ad 5 DNA after treatment by exo III or exo III+S1 Enzymatic digestions were carried out in the following way. To 1 ml ^{3}H-Ad 5 DNA (10^4 c p m μg^{-1}, 300-500 μg ml^{-1} in 0 1×SSC) was added 0 1 ml of a buffer containing 33 mM each of Tris, *p*H 8 0, MgCl$_2$, and 2-mercaptoethanol Digestion was initiated by the addition of 0 1 ml exonuclease III (2000 U ml^{-1}, 1 5×10^5 U mg^{-1} in 40 mM potassium phosphate buffer, *p*H 6 5, containing 1 mM 2-mercaptoethanol and 0 4 M KCl) and incubation at 20° C Exonuclease III was prepared as described[23] with additional purification by phosphocellulose chromatography and Sephadex G100 gel filtration[24] The extent of digestion was monitored by removing 10 μl aliquots and determining the fraction of acid soluble radioactivity At various times 80 μl aliquots were removed and exonucleolytic digestion was stopped by adding 20 μl 0 15 M sodium acetate buffer, *p*H 4 2, containing 5 mM ZnSO$_4$ and 1 5 M NaCl Single-stranded ends were subsequently degraded by adding 20 μl single-strand specific nuclease S1 from *Aspergillus oryzae* (300 U ml^{-1}, 1 5×10^5 U mg^{-1}, in 20 mM sodium acetate buffer, *p*H 4 6, containing 0 1 mM ZnSO$_4$, 0 3 M NaCl, and 5% glycerol) S1 purification was essentially as described by Vogt[25] The reaction was carried out at 37° C for 30 min and was terminated by the addition of 10 μl 0 1 M EDTA, *p*H 8 0 (The % digestion by S1 equalled the amount of DNA made acid soluble by exo III) Finally, HeBS containing 0 5 mM EDTA was added to give a final DNA concentration of 50 μg ml^{-1} and the samples were dialysed against HeBS+0 5 mM EDTA Assays for transforming activity were carried out according to the legend to Fig 1, 2 μg DNA per dish, 5 dishes per point (△) Untreated DNA, (■) exo III treated DNA, (○, ●), exo III-S1 treated DNA *b*, Infectivity and transforming activity of Ad 5 DNA treated with exo III or exo III+S1 Enzyme digestions were carried out as in *a* except that unlabelled Ad 5 DNA was used and the extent of digestion by exo III was determined by measuring the amount of deoxynucleotides incorporated into the treated DNA by DNA polymerase I (purified from an *E coli* pol A mutant deficient in 5′-3′ exonucleolytic activity[23]) Transforming activity (■, ●) and infectivity (□, ○) were assayed as described in the legend to Fig 1 except that infectivity assays were carried out on primary human embryonic kidney (HEK) cells or on a line of HEK cells transformed by Ad 5 DNA ■, □, exo III treated DNA, ●, ○, exo III+S1 treated DNA

Table 1 Calculation of the location of the T segment

Experiment	Fraction of intact single strands	Average no. nicks/strand	Effective no. nicks/strand introduced by exo III*	No. 3′ ends per molecule†	% exo III digestion resulting in 50% reduction in transforming activity	Total % digestion by exo III followed by S1 at 50% reduction	Total % digestion per 3′end (distance from left end of Ad 5 genome to T segment)‡
1	70%	0.36	0.1	2.92	1.3	2.6	0.89
2	32%	1.14	0.1	4.48	2.2	4.4	0.98
3	100%	0	0.1	2.2	1.4	2.8	1.27

The fraction of intact single strands was determined by band sedimentation in alkali in the analytical ultracentrifuge. The average number of nicks per strand was calculated from this fraction assuming a Poisson distribution. The % digestion by exo III followed by S1 was double that by exo III alone.

* The exonuclease preparation had a low level of contaminating endonuclease activity introducing nicks which could then serve as substrates for exonuclease digestion.

† The number of 3′ends per molecule is 2 + the number of nicks per molecule.

‡ Calculated from the total % digestion divided by the number of 3′ ends per molecule.

DNA compared to 18 colonies µg^{-1} for the GC-rich halves.) Thus the T segment appeared to be located in the GC-rich or left half of the Ad 5 DNA molecule, a finding which has been recently confirmed by studies on the transforming activity of DNA fragments obtained by treatment with the R1 restriction endonuclease from *E. coli*.

A more precise localisation of the T segment was obtained by assaying transforming activity of Ad 5 DNA treated with *E. coli* exonuclease III (exo III) (which removes mononucleotides from the 3′ end of polynucleotides in duplex molecules[17]) followed by single strand specific nuclease S1 from *Aspergillus oryzae*. The combination of these two enzymatic treatments has the effect of digesting the Ad 5 DNA molecule inward from the two ends and should result in a decrease in transforming activity when the digestion reaches the region required for transformation.

The results of two experiments are illustrated in Fig. 4*a*. Treatment of Ad 5 DNA with either exo III or S1 separately did not significantly affect transforming activity. The combination of exo III and S1 treatment initially had no effect on transforming activity but after an exo III digestion of about 1% (that is, a total digestion of 2% by the two enzymes), transforming activity dropped rapidly. In contrast, infectivity of Ad 5 DNA was immediately eliminated by exo III–S1 digestion (Fig. 4*b*); digestion of as little as 0.2% (data not shown) resulted in a complete loss of infectivity (> 200-fold decrease) indicating that all molecules were digested by the enzymatic treatment. Figure 4*b* shows also that infectivity was reduced by exo III alone, an effect which is presently being investigated in more detail to determine if, for example, it is related to the inverted terminal repetition contained in adenovirus DNAs[18,19]. The fact that transformation was not immediately affected by exo III–S1 treatment clearly shows that the extreme ends of the Ad 5 DNA molecule are not required for transformation.

We interpret the eventual loss of transforming activity after treatment of Ad 5 DNA with exo III and S1 as being the result of digestion into the T segment and in Table 1 have used the data of Fig. 4 to calculate the distance from the end of the Ad 5 DNA molecule to the beginning of the T segment. When account has been taken for single strand nicks (which are starting points for exonucleolytic digestion) the results from three experiments are in good agreement and give a mean value of 1.1%. From the fact that transforming activity was located on the GC-rich half of the Ad 5 DNA molecule, and from the results of Fig. 3 we conclude that the T segment begins at 1% from the left end of the Ad 5 genome and terminates at about 5–6%.

Implications

Transformation by DNA tumour viruses is often considered to be analogous to lysogenisation of bacteria by bacteriophage, a process thought to involve the integration of the phage genome into the host chromosome by a reciprocal recombination between the host DNA and a circular form of the phage DNA[20]. The results presented in Figs 2 and 3 provide clear evidence that Ad 5 DNA fragments of considerably less than genome size have the ability to transform cells. As it is rather unlikely that such randomly generated fragments can circularise, these results suggest that transformation does not require a circular viral DNA molecule. That fragments as small as 10^6 daltons can transform cells also implies that the proportion of the viral genome which is required to initiate and maintain transformation is less than about 5%. This is in reasonable agreement with results of attempts to determine the size of the transforming region by radiation studies[21], and is consistent with the fact that only about 4–10% of the viral genome is transcribed in rat cells transformed by Ad 2 (ref. 22). Thus, the T segment is large enough to code only for 1–2 average-sized proteins so that presumably not more than about two viral genes are involved in transformation by Ad 5 and these must be contiguous.

We have localised the T segment between 1 and 6% from the left end of the Ad 5 genome. Several lines of evidence are in agreement with this finding. First, Gallimore (P. H. Gallimore, personal communication) has shown that the left end of the Ad 2 genome from 0–14% was always present in several lines of Ad 2 transformed cells. Second, preliminary results indicate that treatment of Ad 5 DNA with two restriction enzymes which cleave in the region between 1 and 6% from the left end results in loss of transforming activity while treatment with a third enzyme which cleaves just to the right of this region leaves transforming activity undiminished and activity appears to be associated with the fragment originating from the left end of the Ad 5 DNA molecule (A. J. van der E., C. Mulder and F. L. G., unpublished).

As in the case of transformation by intact viruses, the mechanism by which the T segment induces cell transformation remains to be elucidated. However, the purification of the T segment, which now appears to be a possibility, should provide a simpler system with which to study the genes and gene products involved in oncogenic transformation.

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letters to nature

Photoelectric photometry of OH471 and OQ172, and other optical objects near radio sources

IDENTIFICATION of the optical objects associated with radio sources is usually made by using the blue and red photographs of the National Geographic Society–Palomar Observatory Sky Survey (PSS) Objects lying near the radio positions are generally counted as possible identifications if they appear diffuse and are therefore galaxies, or appear stellar and blue, and are therefore quasi-stellar object (QSO) candidates Only if radio positions of $\lesssim 1''$ accuracy are available can identifications be made with red or neutral stellar objects without gross contamination of the true identifications by foreground galactic stars, most of which appear neutral or red on the PSS prints

Among radio source identifications hitherto made using accurate (1″) positions there are few red or neutral stellar objects compared with the number of blue ones[1], but they include some of the most interesting of the identifications They include *a* QSOs with redshifts $z \gtrsim 3$, where Lα emission is redshifted to $\lambda \gtrsim 5{,}000$ Å and there are numerous absorption lines blueward of Lα, both effects combining to make the object appear redder than QSOs with lower redshifts, *b* galaxies, which are so compact as to appear stellar on the PSS plates, and *c*, objects like OJ287 which have featureless spectra, often with a redder continuum than those of most QSOs Objects in classes *a* and *b* are usually recognised once their spectra are obtained since those known in these categories have strong Lα emission (class *a*) or strong sharp emission lines of, say, [O II] and [O III] (class *b*) Those in class *c* can only be distinguished from stars on the basis of their featureless spectra which, when the objects are faint, may be a difficult observational task since not all stars show strong absorption lines In this case, *UBV* photometry can confirm the identification through the particular colours of these objects

Radio positions with accuracy ~ 1″ are routinely measured at the University of Texas Radio Astronomy Observatory (UTRAO) and a small percentage of the sources have been identified with red or neutral stellar objects[1] Here, I report on measurements of broadband colours for a small group of the Texas identifications (together with OH471, identified using the same techniques[2]), comparing these with colours of previous radio source identifications I discuss the increased reddening of QSOs with increasing redshift All the identifications are within 3″ of the accurate radio positions, except for one which is within 5″ (Table 1)

U, *B* and *V* magnitudes were measured with two photometer systems on the 82-inch (2 1-m) Struve reflector of McDonald Observatory One system employed a selected RCA 1P21 and the other a RCA 4516 (S20 photocathode) phototube The *U*, *B*, *V* measurements were referred to that system defined by the measurements by Crawford, Golson and Landolt[3] and by Landolt[4] The results of the measurements, and other data, are given in Table 1 Errors given there comprise r m s (random) counting statistical errors added in quadrature to estimated systematic calibration uncertainties The forms of the spectral energy distributions for these objects are shown by their positions in the $U-B$, $B-V$ diagram (Fig 1), where the new data are compared with previous photometry for QSOs, N-galaxies, objects of the OJ287 type, and radio galaxies Here, no corrections have been applied for galactic reddening

It is of interest to compare QSOs in different redshift ranges $z \leqslant 2\ 5$, $2\ 5 < z < 3\ 0$ and $z \sim 3\ 5$ The QSOs having $2\ 5 < z < 3\ 0$ have $U-B > -0\ 4$ compared with $U-B < -0\ 4$ for the lower redshift ones, since for $2\ 5 < z < 3\ 0$ Lα emission occurs predominantly in the *B* filter and absorption shortward of Lα causes a relative decrease of power received in the *U* band The QSO of largest redshift among these objects, 4C05 34 ($z = 2\ 88$, ref 5) has the least negative $U-B$ and can barely be distinguished from an F star on the basis of broadband colours alone[6] Although all the radio-emitting QSOs would have been suggested as QSOs from their blue appearance on the PSS prints, a common criterion employed in suggesting QSO candidates[7–9] has been that $U-B < -0\ 4$ On this basis, all the objects having $2\ 5 \leqslant z < 3\ 0$ would have been missed The two QSOs, OQ172 and OH471, having $z \sim 3\ 5$ (refs 10, 11) have rather larger $B-V$ values than do QSOs of smaller redshift—because of the further redshift of Lα into the *V* passband Note also the large difference (~ 2 mag) in $U-B$ for these two objects at nearly the same *z*—attributable to the much greater absorption below the Lyman limit for OH471 (Spectrophotometry by Oke[12] is in quantitative agreement with these results) OQ172 does appear slightly blue on the PSS prints, whereas OH471 is quite red Thus, on the basis of *UBV* colours or apparent colours from the PSS, QSOs of $z > 3\ 0$ will frequently be indistinguishable from main sequence stars, and objects with greater absorption below the Lyman limit will

Table 1 Details of objects examined

Associated radio source	Other name	Identification¶	Date* February 1974	Uncorrected V	Uncorrected $B-V$	Uncorrected $U-B$	Corrected† weight means V	Corrected† weight means $B-V$	Corrected† weight means $U-B$	Position difference	Position ref	z	Optical spectrum ref
0219+428	3C66A	Like OJ287?	16‡	15 14 ± 0 10	0 48 ± 0 03	−0 38 ± 0 10	14 62	0 30	−0 64	0 7	14	—	14
			20	15 26 ± 0 03	0 52 ± 0 02	−0 45 ± 0 03							
			22	15 18 ± 0 03	0 50 ± 0 02	−0 53 ± 0 03							
0456−043	4C−04 17	GAL	20	16 96 ± 0 08	1 20 ± 0 08	+0 17 ± 0 10	16 56	1 07	+0 07	1 5	UTRAO‖	0 118	UTRAO‖
0642+449	OH471	QSO	22‡	18 49 ± 0 06	1 08 ± 0 10	+1 7 ± 0 4	17 91	0 89	+1 6	2 6	2	3 40	11, 15
0850−034	4C−03 34	RSO	19	17 78 ± 0 05	1 20 ± 0 04	+0 67 ± 0 06	17 37	1 06	+0 57	2 1	1	—	UTRAO‖
1037+302	4C30 19	GAL	20	16 24 ± 0 05	0 98 ± 0 03	+0 16 ± 0 05	16 04	0 91	+0 11	1 3	UTRAO‖	—	UTRAO‖
1049+616	4C61 20	QSO	22	16 48 ± 0 04	0 18 ± 0 02	−0 70 ± 0 03	16 25	0 10	−0 76	1 7	1	0 422	16
1147+245	OM280	NSO	16‡	15 74 ± 0 10	0 52 ± 0 03	−0 60 ± 0 10	15 70	0 43	−0 59	4 9	UTRAO‖	—	15, 16
		Like OJ287?	19§	15 94 ± 0 05	0 39 ± 0 04	−0 49 ± 0 06							
			20	15 89 ± 0 03	0 50 ± 0 03	−0 55 ± 0 02							
1430+095	OQ050	star	16‡	15 76 ± 0 10	1 20 ± 0 03	+1 41 ± 0 10	15 55	1 13	+1 36	2 1	UTRAO‖	—	UTRAO‖
1442+101	OQ172	QSO	22	17 78 ± 0 03	0 80 ± 0 02	−0 37 ± 0 03	17 57	0 73	−0 42	0 4	2	3 53	10
1600+148	4C14 61	RSO	19	18 66 ± 0 15	1 70 ± 0 15	—	18 54	1 76	+10 20	2 0	UTRAO‖	—	UTRAO‖
			20‡	18 92 ± 0 15	1 94 ± 0 10	+0 26 ± 0 20							

Aperture diameter 15″ unless otherwise specified

* On February 16, 20 and 22, 1974 the 1P21 system was used, and on February 19, 1974 the RCA 4516 system

† The following corrections have been applied for galactic extinction[17] In V, −0 18 cosec b^{II}, in $B-V$, −0 06 cosec b^{II}, and in $U-B$, −0 0432 b^{II}

‡ A 7″ diameter aperture was used

§ A 29″ diameter aperture was used

¶ RSO, NSO Red or neutral stellar object

‖ Texas, unpublished

lie even further below the main sequence and to the right, in the $U-B$, $B-V$ diagram Objects of higher redshift will have an even greater proportion of their light output in the V filter A useful criterion for the selection of candidates for QSOs having $z > 3$ may be broadband colours extending to longer effective wavelength, say U, V, R and I, or by comparison of images on ultraviolet, red and infrared plates, analogous to the technique used by Braccesi *et al*[13] to discover lower redshift QSOs

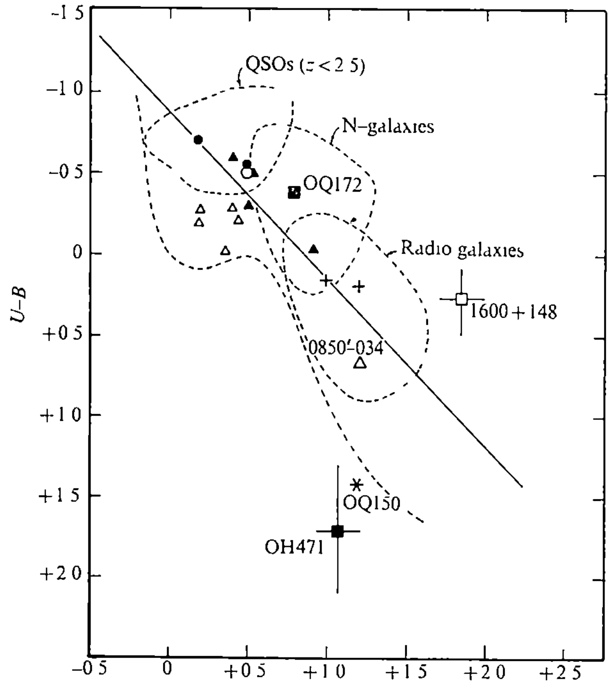

Fig. 1 New colours for some radio source identifications compared with typical colours for QSOs ($z < 2$ 5), N-type galaxies, OJ287-type objects and radio galaxies Error bars shown for OH471 and 1600+148 indicate the dominant contributions from counting statistics The average main sequence and blackbody curves are shown Data for the QSOs PHL957 and 4C05 34 have been taken from Sandage[18], and unpublished data for three other QSOs having $z \sim 2$ 5 have been supplied by R Lynds and D Wills For OJ287-like objects, data are from ref 19 The symbols refer to colours for new QSOs (●), OJ287-type objects (▲), QSOs having redshifts 2 5 < z < 3 0 (△), galaxies (+), and 3C66A (○)

Two of the objects observed (3C66A and 1147+245 (OM 280)) lie in the region of the class *c* objects, above The results for 3C66A have already been discussed[14] No lines have been found yet in the optical spectrum of 1147+245 (refs 15, 16)

Among the red stellar objects, the candidate identification for 1430+095 (OQ050) lies close to the main-sequence curve in Fig 1 and a spectrogram later confirmed that the object is a late type star and therefore probably not associated with the radio source Another red object, within 3″ of 0850−034 (4C−04 17), lies fairly near the main sequence curve, in the region also occupied by radio galaxies Only weak spectra of this object have been obtained by Derek Wills and me, there are certainly no strong emission lines, but the possibility of stellar absorption lines is not excluded The remaining red object, associated with 1600+148 (4C14 61), should probably be classified as a galaxy on the basis of the present photometry Image-tube photographs (scale 25″ mm^{-1}) confirm the stellar nature of the image seen on the PSS, again, however, only weak spectrograms have been obtained ($B \simeq 20$ 5) sufficient only to rule out the presence of very strong emission lines The remaining stellar object in Table 1, 1049+616 (4C61 20), has a redshift[16] $z = 0$ 422 Wills and Wills[16] remarked that the spectrum was somewhat more like that of a galaxy than of a QSO, but the present results show that its colours are quite typical of those of the majority of QSOs

Two galaxies were also observed, they each appear compact (but definitely non-stellar) in the PSS Of these, 0456−043 is the more compact and shows emission lines in the spectrum ($z \simeq 0$ 118), the other, 1037+302 (4C30 19), shows no emission lines The colours of these two objects are quite similar

The present photometry has been valuable in attempting to classify objects on the basis of their continuum colours, especially for the apparently stellar candidate radio source identifications where no lines have been seen in the optical spectrum The colours of the high redshift QSOs, OH471 and OQ172, lead me to suggest that broadband photographic or electrographic photometry at a few effective wavelengths over a wide wavelength range (extending into the infrared) may be a practical alternative to using the properties of radio emission and good radio-optical position agreement in selecting high redshift ($z \geqslant 3$) QSOs, it may therefore be a useful way to test whether or not radio-emitting QSOs are a representative sample of QSOs as a whole

I thank Gerry Grupsmith for observing help and colleagues in the radio group for radio source positions. Unpublished photometry of three QSOs having $z \simeq 2.5$ was supplied by R. Lynds and D. Wills. This work was supported by the National Science Foundation.

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Rapid variations in the spectrum of the magnetic variable star HD215441

THE star HD215441 has the largest magnetic field ever recorded, about 34 Kgauss (ref. 1), varying without change of polarity. There are some indications of smooth brightness variations of this star with amplitude $\Delta m \approx 0.2$ mag as well as of small smooth variations of linear polarisation correlated with the brightness variation[2-4].

Rapid fluctuations (during several minutes) of brightness (W. Schoreich and R. Panov, unpublished) and polarisation in a wide spectral interval as well as polarisation in the wing of the H_β line[6], were also observed. Photoelectric measurements with narrow and wide passband filters showed variations of equivalent width of H_β by 30% within 90 s. Similar variations have not been detected for other Balmer lines[7].

Some efforts to detect rapid spectral variations of the star HD215441 are described below.

Our observations were carried out with a spectrograph and three-stage image tube (with a multi-alkali photocathode) at the Nasmyth focus of the 2.6 m telescope of the Crimean observatory. The dispersion was about 37 Å mm^{-1} at H_α and 46 Å mm^{-1} at H_β, the resolution being about 3 Å. In favourable observing conditions exposure times were 60 s for H_α and 25 s for H_β. During observations on August 25, 26, 28, and September 7, 1973, and January 2, 1974 (when the star was observed during a time interval of more than an hour) more than 300 spectrograms were obtained.

The spectrograms and registrograms show the complicated character of the spectrum with emission (as well as absorption) features. Some successive registrograms of spectra of H_α and H_β regions obtained with a one-minute interval and spectra obtained on different nights are shown in Figs 1 and 2 (the field size of the spectrograph camera does not permit one to obtain H_α and H_β lines on the same spectrogram). The differences in spectra are easily seen. Many details are repeated, but sometimes (for instance on August 26) the emission features in the region of H_α are absent (Fig. 2). Some absorption features of this spectrum correspond to emissions in the spectra of August 28 and September 7. Inspection of the spectra shows that there are continuous intensity variations of many emission features and absorption lines (and probably of the H_α and H_β lines) with the shortest time scale of the order of several minutes.

The complicated character of the spectrum, its variability and low resolution do not permit one to identify emission details with certainty as yet.

Approximate wavelengths of the strongest emissions (determined with an accuracy of 2–3 Å) correspond to FeII, VII, CrII, HfII, TiII, ArII, ClII as frequently as to GdII, LaII, CeII, NdII, SmII. (According to Preston[8], lines of neutral atoms in the spectra, apart from H and HeI, are probably

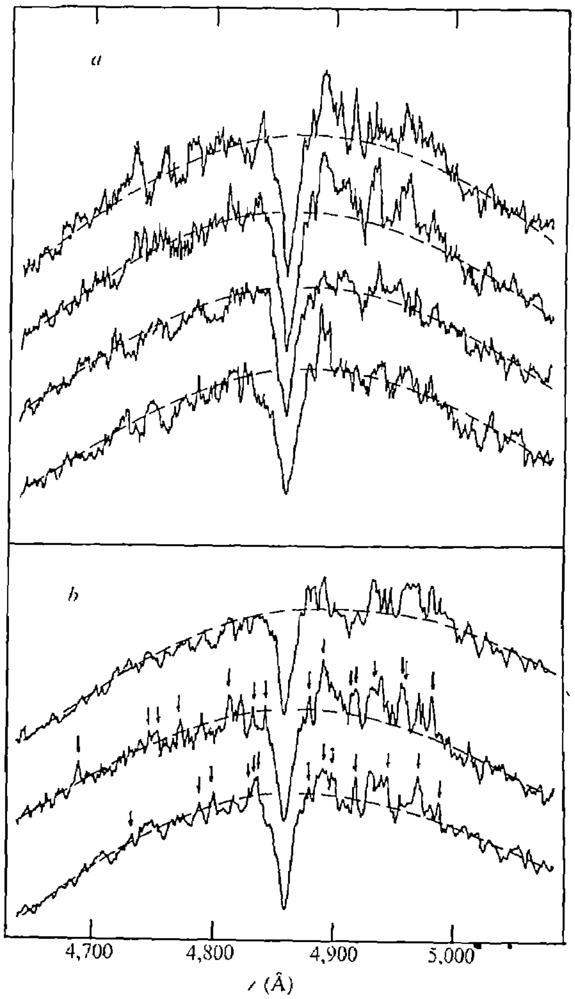

Fig. 1 *a*, Example of registrograms of successive spectra of HD215441, obtained on September 7, 1973, with a one-minute interval in the region of H_β. *b*, Registrograms of spectra, obtained on different nights. Dashed curves show conventional continuum. Top, August 26, 1973, centre, August 28, 1973, bottom September 7, 1973.

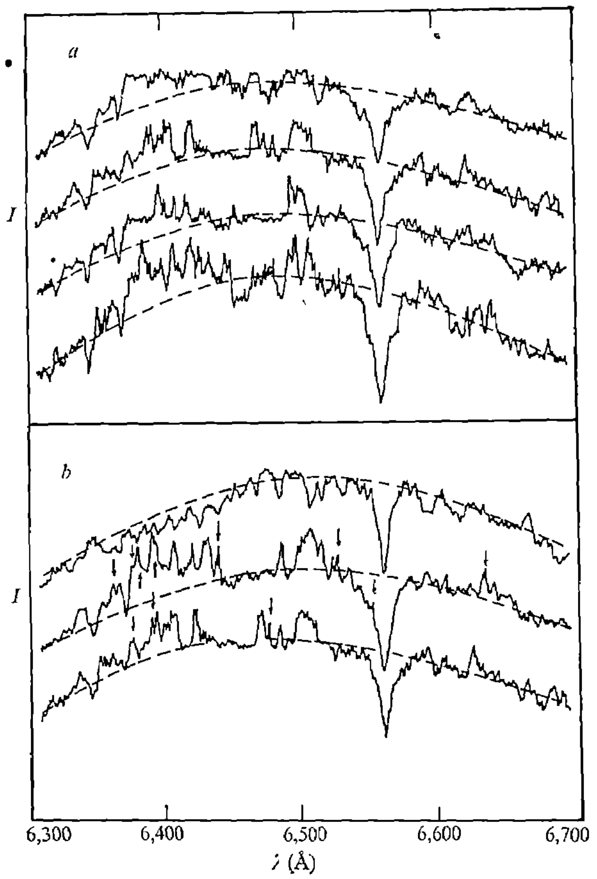

Fig 2 As Fig 1 but for H_α

absent) Positions of some rare earth lines are denoted by arrows on Figs 1*b* and 2*b*

The appearance of rare earth lines in the spectrum of HD215441 is not improbable Extremely variable emission features point to rapid non-stationary processes on the surface of the star, and it is not ruled out that these processes are similar to solar flares In flares the lines of rare earths are sometimes very prominent[9,10] On the other hand, an abnormally high abundance of rare earths is a peculiar feature of some magnetic stars

As some emission details can appear in the wings of hydrogen lines their equivalent widths can change due to blending Thus the equivalent width variations of the H_β line found by Wood[7] might be, more or less, caused by this effect or by a variable emission close to the line

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Photometry of the zodiacal light in the near infrared

During our investigation[1] of comet Kohoutek (1973f) we had occasion to take many photographs of the zodiacal light through various filters In particular we photographed it in infrared light with filters to isolate regions from 610 to 920 nm Later we calibrated the emulsions and camera lenses together by photographing suitably dilute sunlight scattered by a fresh MgO surface

After making allowance for airglow emission it was clear that the albedo of the zodiacal dust cloud is greater, by a factor of two or more, in the region 750 to 920 nm than in the visible This is in contrast to the results of Matsumoto[2] Attempts to observe a reddening of the visible light have been rather inconclusive[3]

Our result is interesting for several reasons We might expect reddening for a very narrow size range of scatters with radii of about 10^{-6}m (ref 4) but this would be disguised if the usually assumed size distributions are correct

The work of Johnson and Fanale[5] on crushed meteorite fragments provides some evidence for a high infrared albedo for asteroidal material (especially for particles of radius $<10^{-4}$ m) but not to the extent that the present observations showed It is difficult to account for this infrared excess without introducing further hypotheses

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High concentrations of ice particles in a layer cloud

Concentrations of ice particles in clouds, greatly in excess of those expected from ice nucleus measurements have been reported[1,2] Most of these observations have been made, however, in cumulus or other complex cloud systems, which increases difficulties in interpretation Here I describe measurements of high concentrations of ice crystals in a simple, nonconvective cloud layer

The measurements were made in very light snowfall (which never exceeded a 'trace' in equivalent precipitation rate) at Snoqualmie Pass, Washington State, between 1045h and 1300h LT on November 28, 1972 The distribution of cloud, winds and temperatures measured with a rawinsonde, and the locations at which airborne and ground measurements were made is shown in Fig 1 The cloud top, as observed from the aircraft, was very smooth and uniform and located at 1,830 m (all altitudes are above mean sealevel) where the temperature was −5° C Cloud base was at 1,220 m, where the temperature was

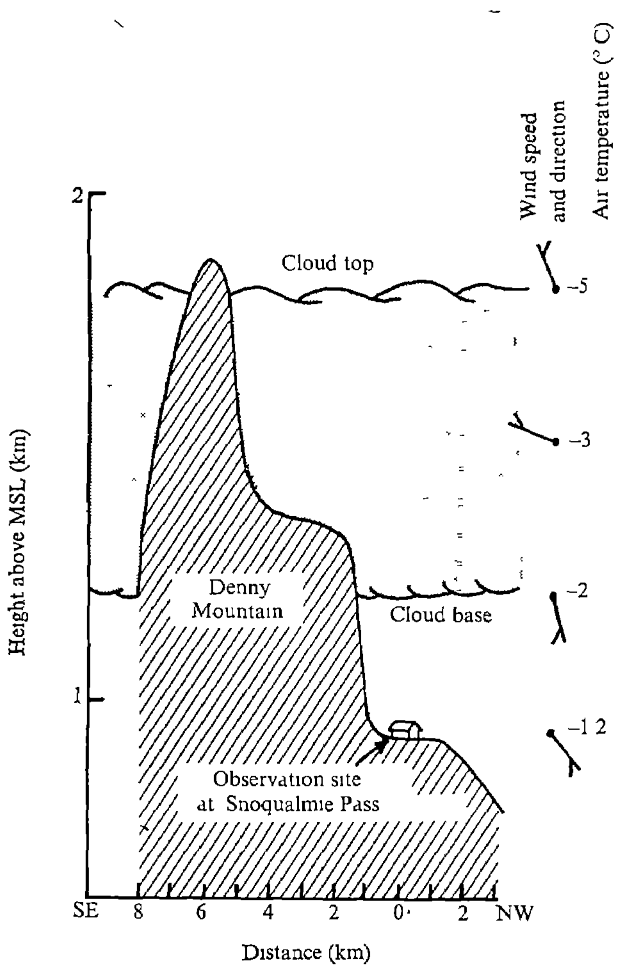

Fig 1 Observation site and atmospheric conditions

−2° C. A spectrum of cloud droplet sizes was obtained near the cloud top with a Formvar cloud particle sampler attached to the aircraft (Fig 2). Also shown in this diagram is the contribution to the liquid water content from droplets of various sizes, droplets with diameters up to 75 μm were present.

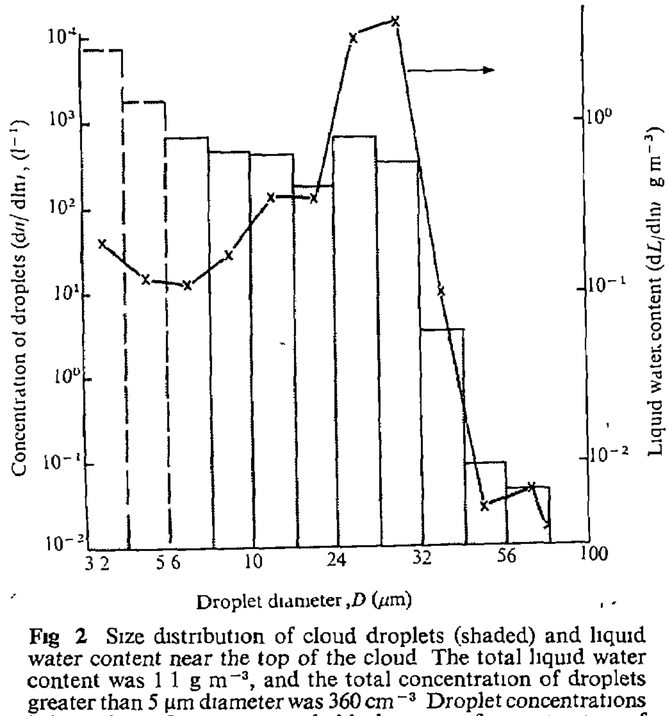

Fig 2 Size distribution of cloud droplets (shaded) and liquid water content near the top of the cloud. The total liquid water content was 1.1 g m^{-3}, and the total concentration of droplets greater than 5 μm diameter was 360 cm^{-3}. Droplet concentrations below about 5 μm are unreliable because of uncertainties of collection

Some isolated ice needles and irregular crystals were also collected from the aircraft flying near the cloud top.

The crystals reaching the ground site (elevation 915 m) initially consisted of single needles and sheaths, but as the snowfall rate increased they formed aggregates. As the number of crystals in an aggregate is difficult to estimate, the results described here are confined to the period when single crystals only were collected. The concentrations of single crystals in the air were deduced from measurements of the number of crystals which fell on to glass slides exposed horizontally for measured time intervals, by considering the speeds of fall of needles and sheaths ($\simeq 1$ m s^{-1})[3]. The concentrations of crystals in the air was about 1 l^{-1}. Measurements of atmospheric ice nuclei indicated that at the minimum temperature in the cloud (−5° C), the concentration of ice nuclei should be about 10^{-4} to 10^{-3} l^{-1}. Therefore, the concentrations of the crystals in this simple layer cloud exceeded, by at least a factor of 10^3, those expected from measurements of ice nuclei.

The size distributions of the crystals collected on the ground are shown in Fig 3. The crystals tended to be either completely unrimed or moderately to densely rimed. The droplets frozen on to rimed crystals were up to 100 μm in diameter and were concentrated on the leading edges of the crystals. The rimed crystals tended to be fewer in number but larger in size than the unrimed crystals, indicating that the unrimed crystals had shorter growth times. The unrimed crystals possibly originated on ice splinters produced during riming in the lower regions of the cloud. In this connection, we note that the conditions under which riming occurred in this cloud encompass the critical combination of conditions for temperature, droplet sizes and impact speeds under which large numbers of splinters have been observed in laboratory experiments[4]. As unusually high concentrations of ice crystals, however, have been observed in clouds where there was no riming (G. Vali, personal communication) and, in some situations, do not occur in clouds where riming does take place[5], there is still no completely satisfactory theory to explain the phenomenon.

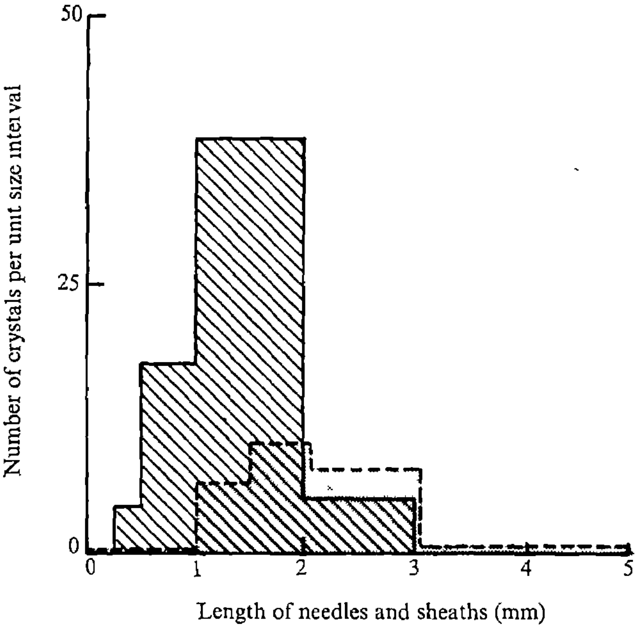

Fig. 3 Size distribution of rimed (shaded) and unrimed (hatched) needles and sheaths collected at the observation site

The high concentrations of ice crystals which occurred in the cloud described here could not have been produced by the mechanism postulated by Mason[6] because that requires convective towers. Indeed, contrary to Mason's contention, there are several sets of independent field observations which show high concentrations of ice crystals in non-convective clouds (refs 2 and 7, and G Vali, personal communication)

Because there are natural limits to the masses and speeds of fall which ice crystals can achieve in the atmosphere, ice crystal concentrations of the order of 1 l^{-1} are needed to produce even light snowfalls Such concentrations must, therefore, be quite common in shallow layer clouds For example, a needle crystal of 5 μg mass has a speed of fall of about 1 m s^{-1} To produce an equivalent water precipitation rate as small as 0 02 mm h^{-1}, these crystals have to exist in the air in concentrations of about 1 l^{-1}

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Mercury in the sediments of the Pallanza Basin

SURFACE sediment samples (0–3 cm) taken from the Pallanza Basin of Lago Maggiore, Italy, in 1970 have been analysed for mercury, using flameless atomic absorption spectrophotometry The concentration of mercury in the Maggiore sediments proved to be extremely high when compared with other reported lake sediments (Table 1, and ref 1) They were checked by another laboratory and both sets of results were in extremely good agreement Mercury concentrations ranged from 0 2 to 20 1 p p m, with a mean of 7 7 p p m and a standard deviation of 4 9 p p m

The highest concentrations of mercury in the Pallanza Basin occur to the south of Pallanza, with additional elevated regions occurring in the nearshore area to the north of Stresa and off Feriolo (Fig 1*a*) Thomas[2] has demonstrated the use of a quartz correction for total mercury in assessing the variable adsorption of mercury by sediment fines and in locating point source inputs A similar correction has been applied for the Pallanza Basin sediments, in which total observed mercury has been corrected for the percentage of material of sand size (Fig 1*b*) Mercury enriched sediment occurs predominantly along the north shore of the basin, in water which is up to approximately 125 m deep, with the Toce River as the indicated primary source The areas of higher concentration at Feriolo and Stresa are again apparent from the distribution given in Fig 1*b*

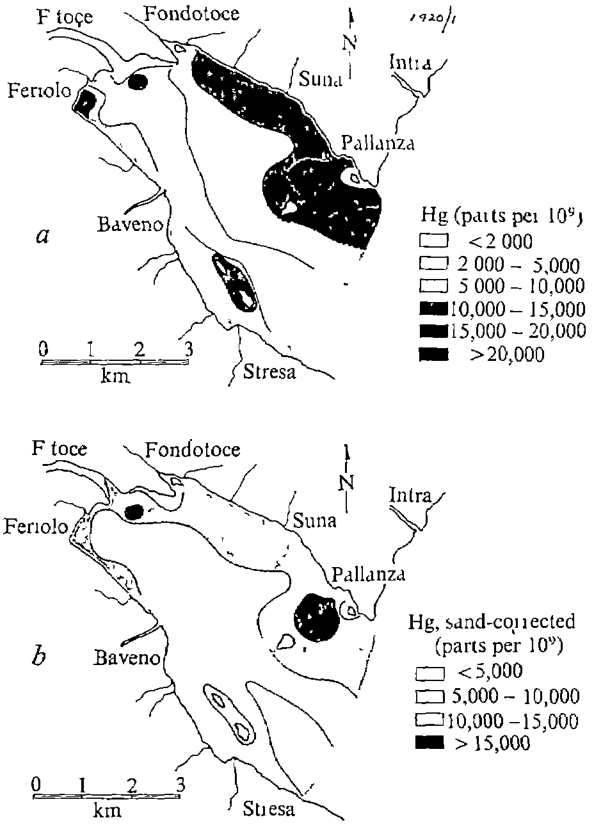

Fig 1 *a*, Distribution of total observed mercury in the surface sediments of the Pallanza Basin, *b*, distribution of sand corrected mercury in the sediments of the Pallanza Basin

After the start of summer stratification, in late March, suspended materials associated with Toce River waters occur predominantly to a maximum depth of 50 m in the Pallanza Basin[3,4] Thus, through the bulk of the basin, turbid Toce River water flows over the hypolimnion and is transported to the open waters of Lago Maggiore In the shallow water areas, suspended solids, associated predominantly with the epilimnion, are deposited and retained The maximum input of solids from the Toce River takes place with the spring runoff which occurs in late April and May, postdating the start of lake stratification[4]

Table 1 Mercury concentrations in some lacustrine, surface sediments*

Lake	No of samples	Depth of sample (cm)	Concentration (parts per 10^9) Minimum	Maximum	Mean	Standard deviation
Superior	405	0–3	4	584	83	56
South Michigan	31	Variable	30	380	146	
North Channel	55	0–3	8	1,112	151	231
Huron Georgian Bay	117	0–3	12	9,500	257	1,013
Huron	163	0–3	54	805	222	162
St Clair	55	0–2	70	2,565	632	628
Erie	243	0–3	13	7,488	609	703
Ontario	248	0–3	32	2,100	651	507
Pallanza Basin, Lago Maggiore	34	0–3	240	20,100	7,649	4,882

* Data from ref 1

The relationship of total mercury to other sedimentary parameters has been examined by means of a linear correlation matrix Significant relationships were observed with the clay size fraction ($r = 0\ 722$) and with iron ($r = 0\ 553$), suggesting that the mercury is associated with iron rich, detrital clay particles (see ref 5) No significant relationship with organic carbon ($r = 0\ 367$) was observed

The impact of the high mercury concentrations on the aquatic biota, has not been examined Damiani[6] discussed the variation of the C/N ratio in the sediments of the basin and noted that lower ratios, attributable to autochthonous organic matter, occurred in the shallow water areas along the north shore from Fondotoce to Pallanza, north of Stresa and off Feriolo Higher C/N ratios, indicative of allochthonous organic matter, were observed in the deeper central waters of the Pallanza Basin and were attributed to direct derivation from the Toce River Figure 2 shows the relationship of mercury to the C/N ratio, the relationship is significant, with a correlation coefficient of $-0\ 656$ Even though the gross statistical relationships show that the mercury is associated predominantly with the clays, the relationship of mercury with the C/N ratio implies that the mercury may in part, be associated with autochthonous organic matter This is in distinct contrast to the lower mercury levels in allochthonous organic matter with a high C/N ratio, derived from the Toce River It is possible that this relationship is coincidental in that the deposition of clay and associated mercury from the Toce River occurs predominantly in the shallow water regions which in turn receive the highest input of deposited autochthonous organic matter We feel, however, that the degree of the relationship between mercury and autochthonous organic matter, as inferred from the C/N ratio, implies uptake of mercury by the primary producers in the shallow water zones, which indicates that mercury transfer processes are taking place Confirmation may follow from an examination of the levels of mercury concentration in the biota of the lake

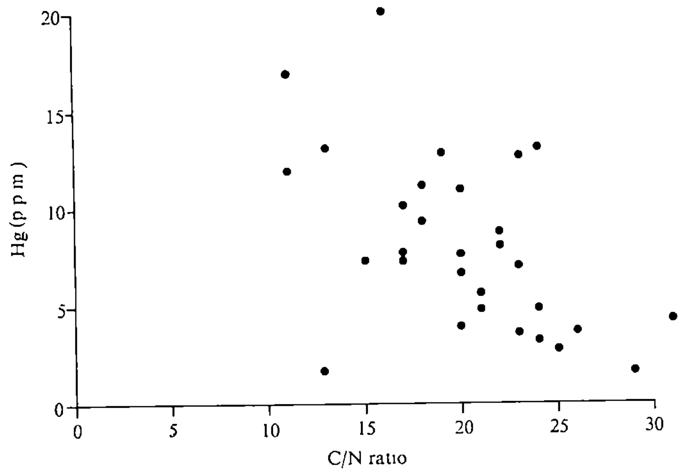

Fig 2 Relationship of mercury to the C/N ratio in the sediments of the Pallanza Basin

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Drag reduction in polymer mixtures and the effect of molecular weight distribution

Small amounts of certain high molecular weight polymers can reduce the frictional drag in turbulent flow through pipes[1,2] Although the phenomenon—the so-called drag reduction effect—has been studied extensively, very little information is available about the effects of molecular weight distribution in polydisperse samples All common drag reducing agents are characterised by a broad distribution of molecular weights, which changes during shear degradation[3,4] Consequently, any molecular theory of drag reduction must include some means of handling these complications The equations presented here represent a possible first step in this direction

For many materials the percentage of frictional drag reduction at a fixed Reynolds number, DR, can be related to a reduced polymer concentration, r, by the empirical equation[5,6]

$$DR/DR_{\max} = r/(1+r) \qquad (1)$$

where

$$r = c/[c] \qquad (2)$$

The polymer concentration, in weight parts per million (w p p m), is represented by c, and $[c]$ and $DR_{\max}$ are empirical constants called the intrinsic concentration and the maximum drag reduction, respectively These equations are valid at flow rates significantly above the onset of drag reduction and at relatively low concentrations, where DR is substantially less than $DR_{\max}$ At higher concentrations the measured drag reduction is often less than that predicted by equation (1) and thus $DR_{\max}$ is seldom obtained in experiments This is parti-

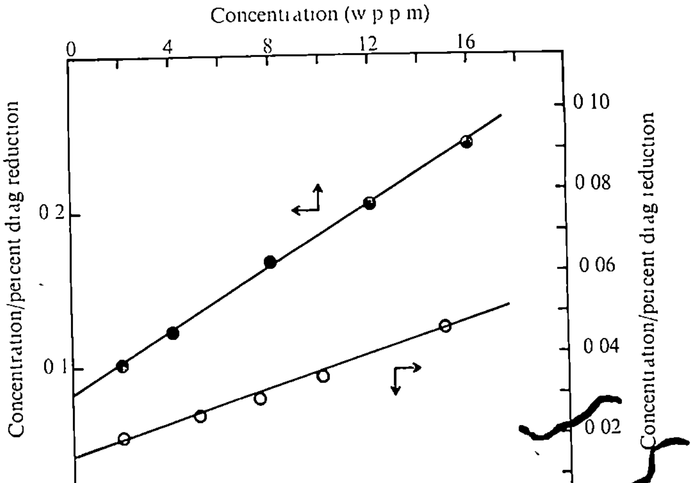

Fig 1 Graph of concentration over percent drag reduction against concentration ●, WSR-205 data, ○, WSR-301 data Slope equals ($1/DR_{\max}$), intercept on the ordinate equals ($[c]/DR_{\max}$)

Table 1 Properties of polymer samples

Compound	[c]	DR_{max}
WSR-205	8 0	98
WSR-301	1 5	98

cularly noticeable in capillary tube measurements where DR_{max} can be quite large The intrinsic concentration is a measure of the polymer's effectiveness as a drag reducing additive, that is, materials with the lowest values for [c] are the most effective

Little, Ting and Kim have studied the drag reducing ability of polyethylene oxide[6] and polyacrylamide (unpublished work) They found that equations (1) and (2) are obeyed at low concentrations and that [c] can be related to the weight average molecular weight, $\bar{M}$

$$[c] = A/(\bar{M}-M_0) \qquad (3)$$

where A is a constant characteristic of the polymer family and M_0 is the molecular weight below which no drag reduction is observed in that family The weight average molecular weight can be defined as

$$\bar{M} = \sum_i c_i M_i / c \qquad (4)$$

where c_i is the concentration (w p p m) of molecules with molecular weight M_i Equations (1) and (2) may now be written as

$$\frac{DR}{DR_{max}} = \frac{\sum_i r_i}{(1 + \sum_i r_i)}$$

where r_i is the reduced concentration of the ith component

$$r_i = c_i/[c_i] \qquad (6)$$

and $[c_i]$ is the intrinsic concentration of the ith component

$$[c_i] = \frac{A}{(M_i - M_0)} \quad \text{when } M_i > M_0 \qquad (7)$$

$$[c_i] \rightarrow \infty \text{ when } M_i \leqslant M_0$$

Experimental results[6] also indicate that DR_{max} varies with molecular weight but only for molecules where M_i is close to M_0 If $\bar{M}$ is significantly greater than M_0, the effects of these lower molecular weight molecules is small and so DR_{max} can be treated as a constant

These results suggest that reduced concentrations should be additive when the sample is considered to be a mixture of many different components, each with a single molecular weight The best way to test these equations would be to study mixtures of several different monodisperse polymers from the same polymer family Unfortunately, such samples are very difficult to obtain An alternate but less satisfactory procedure is to test mixtures of polydisperse samples Expressions for drag reduction with mixtures of polydisperse samples from the same polymer family can also be derived using equations (2–4) The results are similar to equations (6) and (7) except that M_i is replaced by $\bar{M}_i$, the weight average molecular weight of the ith component This means that the reduced concentrations should be additive for mixtures of polydisperse samples as well as for mixtures of monodisperse samples

Table 2 Measured and predicted* drag reduction in mixtures

WSR-205 concentration (w p p m)	Drag reduction at indicated concentration (w p p m) of WSR-301						
	0	0 25	0 5	1 0	1 5	2	3
0			24(25)	40(39)	52(49)	59(56)	65(65)
0 5		19(18)	27(26)	41(41)	51(51)		
1 0		22(22)	30(31)	43(43)	54(52)		
2 0	20(20)	29(29)	34(36)	47(47)	55(54)		
4 0	33(33)	36(39)	44(45)	53(53)			
8 0	48(49)	52(53)	57(56)				
12 0	59(59)						
16 0	66(65)						

*Values predicted from equations (6) and (7) are in parentheses

To test this, two high molecular weight polyethylene oxide samples were obtained (Union Carbide Corp) An automated capillary rheometer[7] was used to measure the concentration dependence of drag reduction for aqueous solutions of both samples at a Reynolds number of 9,000 The capillary tube has an inside diameter of 0 1575 cm The intrinsic concentrations and maximum drag reductions were then determined[6] from the slopes and intercepts in graphical plots of c/DR against c Figure 1 shows graphs for the two samples, and in both cases the linearity is excellent The values of [c] and DR_{max} are listed in Table 1 Various mixtures of these two samples were then prepared and their drag reduction measured at a Reynolds number of 9,000 The results are listed in Table 2

In each case the predicted and experimental results are in agreement in spite of the fact that these mixtures represent a variety of distinctly different average molecular weights and molecular weight distributions This suggests that equations (5–7) may represent a meaningful first step towards understanding the behaviour of mixtures and, therefore, the effects of molecular weight distribution It is hoped that, as monodisperse drag reducing polymers become available, these relationships can be more rigorously tested

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Role of upper-layer interactions in electron diffraction symmetries

STEEDS *et al* have published pictures showing effects of upper-layer interactions in molybdenite[1,2] Because of its use in structure analysis it seems worthwhile to set down rules from the literature which relate electron diffraction symmetries to three-dimensional crystal symmetries As distinct from problems which can be solved by considering only zero-layer interactions, such as the determination of a polar axis in the plane of projection[3,4], the total crystal symmetry, including the boundaries, must be considered when upper-layer interactions are important As a simple example some results obtained from a highly tilted crystal are given, and I also comment on the conclusions of Steeds *et al*[1,2]

There are two rules pertaining to the symmetry of the zone-

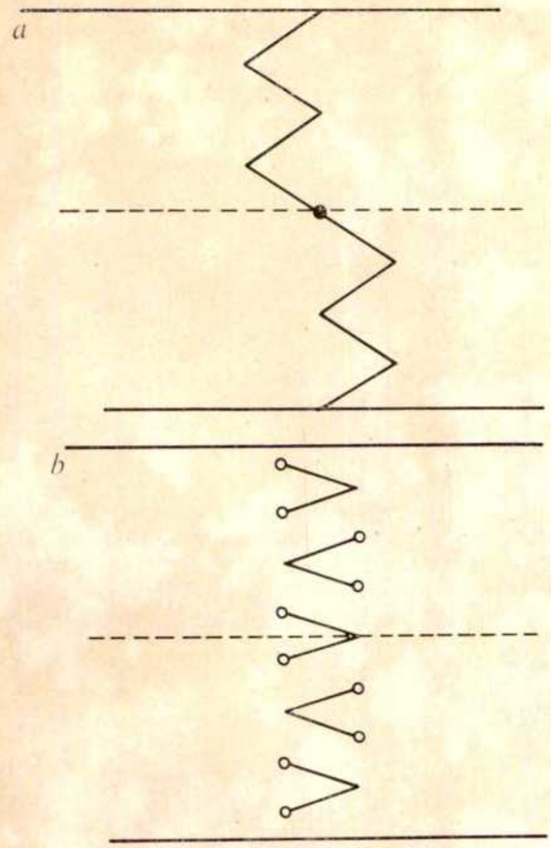

Fig. 1 The significance of a central mirror plane to the zero-beam diffraction symmetry. *a*, Representation of a crystal with the stacking sequence A-B-A-B-A-C-A-C-A. This illustrates the case of a graphite crystal with a central stacking fault. This crystal has no central mirror plane or glide plane, although it has a centre of symmetry[12]. The zero-beam distribution resulting from diffraction will be non-centrosymmetric. *b*, Representation of a molybdenite crystal of five layers ($2\frac{1}{2}$ unit cells), which has a central mirror plane but not a centre of symmetry. The zero-beam distribution will be centrosymmetric. Empirical results are dependent on structure (Table 1). Ray diagrams, incorporating the law of reciprocity, given in publications referenced in the text, make these conclusions obvious.

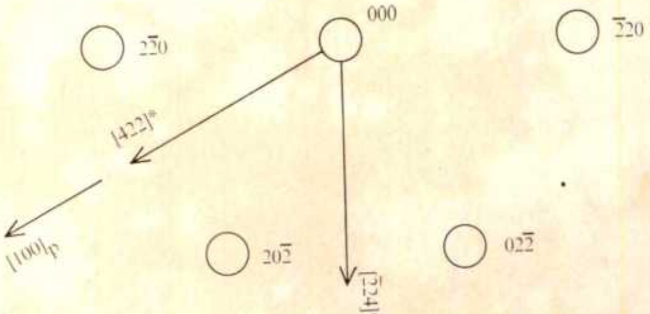

Fig. 2 Indexing of reflections relative to the projection of the surface normal, [100] p, for the [111] pattern; $02\bar{2}$, $02\bar{2}$, are zero-layer reflections. Other 220-indexed reflections, using the present nomenclature, belong to first upper and lower layers.

axis pattern[3,5,6]. First, the condition for a centre of symmetry in the zero-beam pattern is that the crystal as a whole has a mirror or glide plane, perpendicular to the zone axis, midway between its surfaces. Second, an additional condition for a centre of symmetry in the remainder of the diffraction pattern is that there is a centre of symmetry in the two-dimensional projection of the crystal.

These conditions ((1) and (2)) relate, respectively, to upper-layer and zero-layer interactions. The latter terms need definitions appropriate to dynamic scattering. In discussions of X-ray crystallography 'upper layer' and 'zero-layer' are used for a particular zone independent of the scattering experiment, and/or an infinite crystal (thus, crystal boundaries are irrelevant to this nomenclature). It is much better to use another nomenclature for electron diffraction.

'Zero-layer reflections' are defined here as those reflections generated by scattering from the z-independent structure, that is, from the finite crystal with a structure averaged out in the z-direction between the crystal surfaces, where the z-direction is identified with the surface normal. This definition, following the scattering process rather than the unit-cell geometry, allows 'upper-layer interactions' to be associated with effects strictly connected with propagation. This is consistent with established theory[7-9].

Condition (1) can only be fulfilled for a parallel-plate crystal when the surface normal is parallel to the zone axis. This condition refers to the symmetry of the crystal as a whole rather than simply to the space-group symmetries of the unit cell. Condition (1), and thus the zero-beam symmetry, has no direct bearing on the question of whether the crystal as a whole, or the unit cell, has a centre of symmetry (Figs 1*a*, *b*, and 2). Both conditions taken together determine a centrosymmetric crystal. The two conditions can, however, be exploited separately in analyses (Table 1).

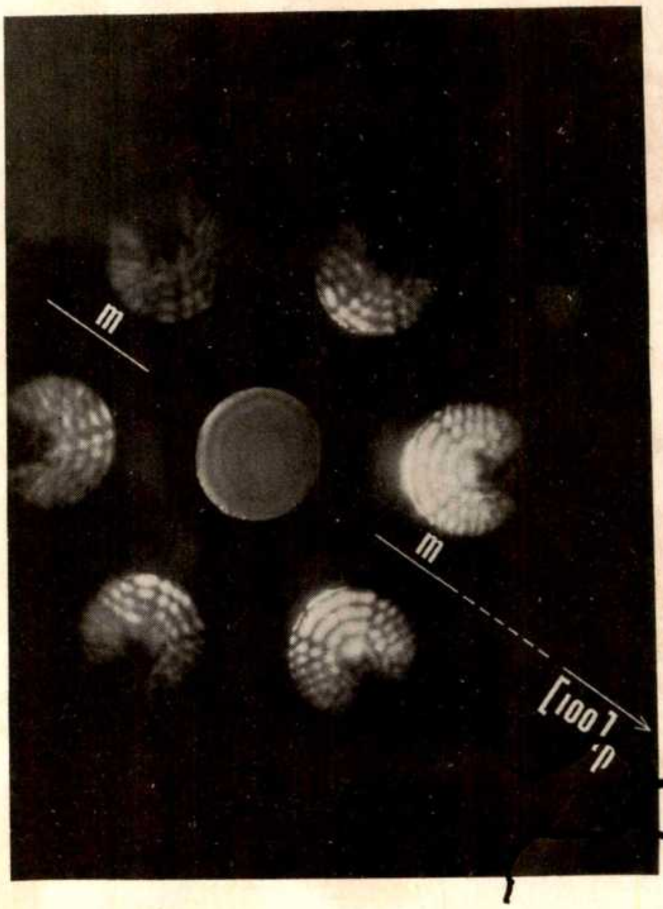

Fig. 3 Convergent-beam diffraction pattern from an MgO platelet with (100) faces, at the [111] zone axis, using 80 kV electrons. Direction of the projection of [100] is indicated. The only symmetry in the diffraction pattern including the zero beam is a mirror line perpendicular to this direction.

Table 1 Effect of structure on empirical results

Crystal symmetry		Diffraction symmetry Zero beam	Diffraction symmetry Rest of pattern
(1) Centrosymmetric crystal	Central mirror plane	Centrosymmetric	Centrosymmetric
	No central mirror plane (examples: Figs 1*a* and 2)	Non-centrosymmetric	Non-centrosymmetric
(2) Non-centrosymmetric crystal, centrosymmetric in projection	No central mirror plane	Detectably non-centrosymmetric only for a large (polar) *c*-axis, or long disorder parameter in the c direction	
(3) Crystal non-centrosymmetric in projection	Central mirror plane (example: CdS, $BaTIO_3$, space group symmetry, Fig. 1*b* (crystal boundaries)	Centrosymmetric	Non-centrosymmetric
	No central mirror plane	Detectably non-centrosymmetric as for (2)	Non-centrosymmetric

As an example in which upper-layer interactions strongly influence the symmetry of the diffraction pattern, consider the [111] zone-axis pattern obtained from an MgO plate with (100) surfaces. I reason that the hexagonal symmetry of a crystal with [111] surfaces will be destroyed by upper-layer interactions. The pattern will only have one mirror line, through its centre, parallel to the set of mirror planes of the whole crystal, that is, parallel to planes containing [100] and [111] (Fig. 3). The pattern symmetry corresponds directly to the three-dimensional symmetry of the crystal in that orientation.

The effect of symmetry on the zero-beam can be increased by tilting the crystal away from the axis, which increases the dynamic coupling. Figure 4 shows the convergent-beam picture for the 'three-beam pattern' near $\zeta_{02\bar{2}} = 0$ and $\zeta_{20\bar{2}} = 0$. With this indexing the projection of the surface normal, [100], is parallel to $[4\bar{2}\bar{2}]^*$. As the space-group mirror line $[22\bar{4}]$†, bisecting the two scattering vectors, is not contained in a mirror plane of the whole crystal, the two reflections have unequal intensity distributions. The asymmetry in the zero-beam distribution about this line demonstrates the upper-layer origin of this effect. An almost perfect mirror line is, however, observed through the $02\bar{2}$ distribution along the line $\zeta_{02\bar{2}} = 0$. This line is parallel to the mirror plane of the crystal.

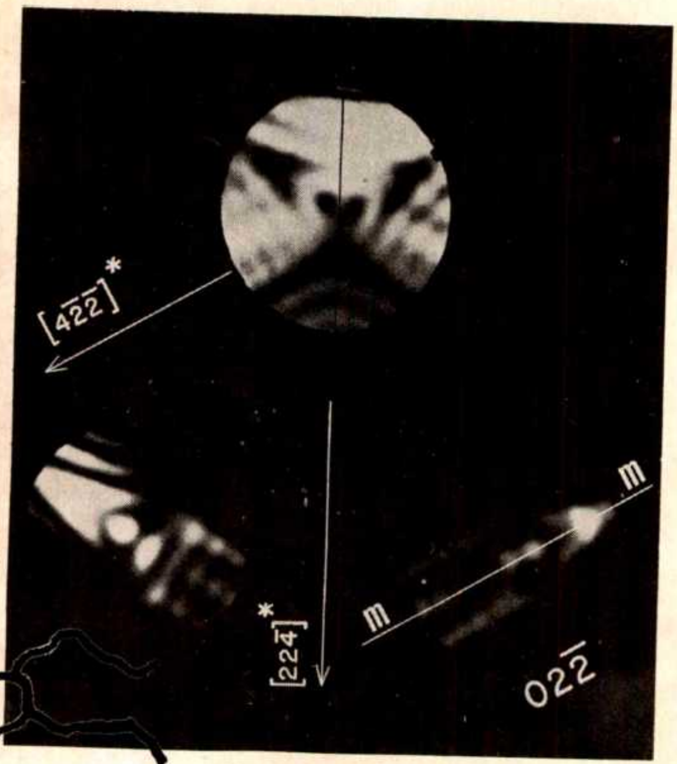

Fig. 4 Convergent-beam diffraction from the crystal used for Fig. 3 tilted to satisfy the $02\bar{2}$ and $20\bar{2}$ reflections. With this indexing the projected [100] surface normal is parallel to $[4\bar{2}\bar{2}]^*$, which is also parallel to the mirror planes of the crystal. The $02\bar{2}$ reflection is symmetric about the line m—m of zero excitation error.

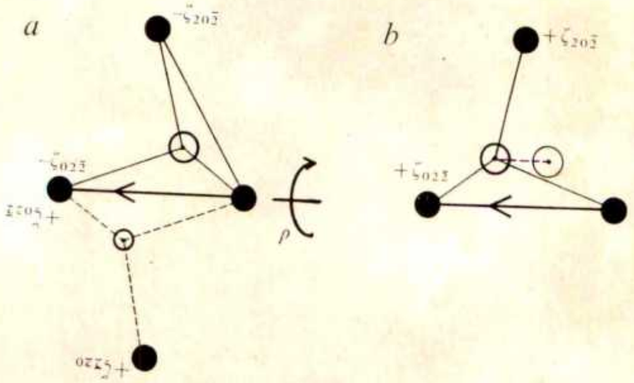

Fig. 5 *a*, The reciprocity construction of Moodie[4] projected on to the plane perpendicular to [111]. The centre of the Ewald sphere is shown encircled; arrow, scattering vector for $02\bar{2}$. The reciprocally related diagram, below the plane of projection, is indicated by broken lines, and its excitation errors and their signs are shown inverted. *b*, Result of rotating the reciprocal diagram of *a* through 180° about the scattering vector. The original Ewald sphere centre is also shown, indicating that its displacement is parallel to the diffraction vector.

It is important to ascertain whether symmetries apparent in the pattern are expected to be exact, or only approximate, theoretically, because the former case provides a good test of experimental accuracy. The construction given by Moodie[6] for the application of the reciprocity theorem allows determination of the symmetry of the $02\bar{2}$ reflection for this crystal. This construction gives the two incident directions leading to identical scattering vectors and amplitudes for any crystal (Fig. 5*a*). Equivalent incident directions are obtained by operations on the diagrams which, because of symmetries, leave the crystal unchanged. To exclude the trivial exercise of equating intensities from crystallographically equivalent zone axes, these operations must leave both crystal and zone axis unchanged.

Equivalent conditions can be found for the $02\bar{2}$ beam by rotating the diagram through 180° (Fig. 5*b*). This operation will leave the crystal unchanged provided it is centrosymmetric (that is, if conditions (1) and (2) are fulfilled) (Fig. 6). Figure 5 gives proof of the existence of a mirror line in the $02\bar{2}$ distribu-

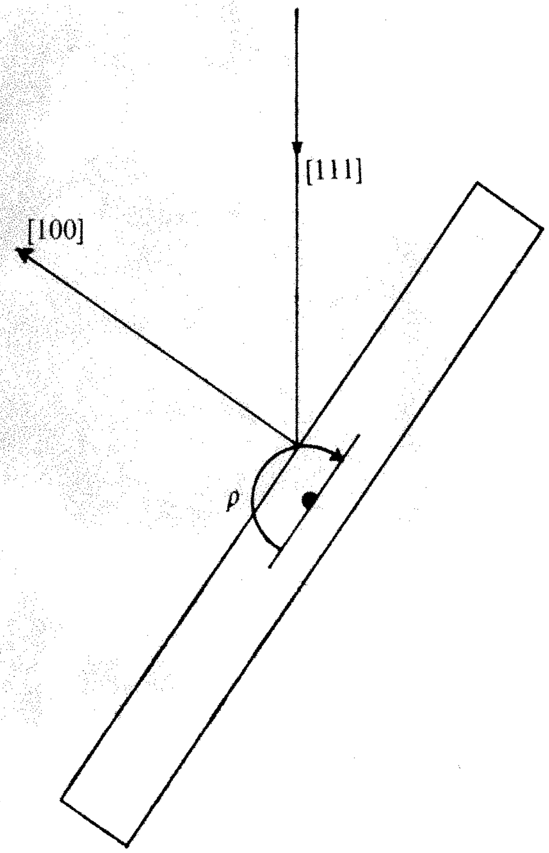

Fig. 6 Diagram showing the tilted crystal. Relationship between surface normal [100], zone axis [111], and the 180° rotation ρ about the 02$\bar{2}$ scattering vector are shown.

tion, for the centrosymmetric MgO structure. The setting of Fig. 4 thus provides the additional information necessary for interpretation of Fig. 3: the asymmetry at the zone axis arises from the influence of crystal boundaries and not from a polar structure. This conclusion follows directly from the proof of Fig. 5.

There are published observations of the effects on symmetry of stacking faults[10]. In MoS_2 the most common cause will be a S/S shear[11] somewhere central in the crystal. The problem is closely related to that in graphite, for which complete calculations exist[12]. In the case of a pure rhombohedral crystal there is a regular rather than an irregular sequence of basal faults, over a finite thickness. I believe that distinguishing these cases from the data, and locating the positions of faults, requires numerical analysis, and that the rules quoted by Steeds *et al.*[2] are not always followed. As extreme examples I refer to the upper-layer unit cell (regular stacking) effects in gold[13]. In these cases there are antisymmetric contours over relatively large angles away from the [111] zone. There is also a strong trigonal pattern caused by a single stacking fault in graphite[12].

Diffraction contoured micrographs allow exploration of a large angular region using one diffraction order, but lack linear mapping and fine specimen resolution, as crystal curvature cannot be controlled. On the other hand, convergent-beam diffraction allows specimen resolution down to 50 Å, with accurate angular resolution, but over a necessarily limited angular region. Wide-angle convergent-beam patterns[14] (with overlapping diffraction orders) can be shown to have arbitrarily imposed symmetries, although a series of normal Kossel-Möllenstedt patterns (non-overlapping orders) can be used to map out the entire region of the zone axis. As specimen resolution is, however, limited, this is not a universal technique, and normal electron micrographs are also required. The relative advantages of either of the conjugate objective planes for structure analysis will be discussed in future (W. C. T. Dowell, and P. Goodman, unpublished).

The wavelength dependence of patterns of the zone axis provides a simple test[15] of the structure factor as distinct from shape-transform[6] dependence. It is misleading, however, to refer to the two-beam approximation in connection with the low-voltage (or large excitation error) zone-axis pattern, and more correct (A. F. Moodie, unpublished) to refer to a different approximation (for example, the second Born approximation, or the thick phase-grating[16] approximation).

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DR STEEDS REPLIES: Dr Goodman[1] casts doubt on the bright field rotation method[2] of identifying the presence of faults parallel to the surface of a specimen. The examples quoted in support of his point of view are, however, inadequate. Whereas our experiments are often performed over rotations of 20° or more, in the convergent beam work in gold, cited by Dr. Goodman, the angular range in view is 1.29° at 4.21° from [111], which is much too limited for a test. During large rotations the systematics-only pattern is interrupted by various non-systematic reflections, but there are several ranges of orientation in which non-systematics are not important and the asymmetric pattern returns to essentially the same form. The graphite example is irrelevant because the field of view is restricted to a narrow angular range close to the [0001] zone axis where the pattern is dominated by rapidly varying cross-grating contributions; our 'rule' refers to contours which result essentially from one-dimensional diffraction. Clearly, the misunderstanding arises from the very great difference of angular view in convergent beam and bend contour patterns, despite their formal equivalence (to a first approximation). Effects of inclination, although important for really large tilts, such as the 54° from the normal of the foil used by Dr Goodman, are not in our experience significant for smaller rotations of the order which we propose in our experimental test, at least not for bend contour patterns, where a certain amount of distortion is almost inevitable.

Dr Goodman claims that our reference to "two-beam-like behaviour" is misleading, and so we wish to state explicitly what was meant by that phrase. It is possible to show from the many-beam theory of electron diffraction that (B. Buxton, unpublished)

$$\frac{d\xi_{ij}}{d\beta} = \xi_{ij}\left\{\frac{1}{\beta}+\beta\gamma^2\left[1-\frac{\gamma}{s_i-s_j}\frac{d(s_i-s_j)}{d\gamma}\right]\right\}$$

$$s_i-s_j = (\gamma^{(i)}-\gamma^{(j)})/4\pi k$$

where $\gamma^{(i)}$, $\gamma^{(j)}$ are the eigen values obtained by matrix diagonalisation (usual notation[3]); ξ_{ij} is the extinction distance corresponding to $(i-j)$ dispersion surface transitions; $\beta = v/c$; and $\gamma = (1-\beta^2)^{-1/2}$.

According to strict two-beam theory $d(s_i-s_j)/d\gamma = (s_i-s_j)/\gamma$ and so our result holds. There are, however, a number of other situations, which we call "two-beam-like", in which the second term in the above expression vanishes or is negligibly small. These often occur when there are only two branches of the dispersion surface with excitations which vary rapidly with orientation (characteristic low voltage behaviour). Even in extreme cases of many-beam diffraction, such as in the case of molybdenite in our illustration, the pattern can be regarded as coming largely from transistions between two branches, although the branches involved change with orientation.

We find Dr Goodman's change of definition of zero and upper layer reflections unnecessary and inconvenient[4], involving as it does the surface normal. In our experience, the relative orientation of a zone axis and the surface normal may stray by 20° or more without significantly affecting zone axis patterns. Finally, although Dr Goodman's two conditions are sufficient they are not necessary. To take an extreme case not covered by condition (1) we may obtain a centre of symmetry in the zone beam pattern by diffraction from two different monolayers having centres of symmetry colinear with the mean direction of the incident beam (we are grateful to Professor Frank for making this point).

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A new penetrating-radiation image converter

A NEW practical penetrating-radiation television camera tube, termed the Metallonicon, has been built. It operates at X-ray frequencies, and a discernible image is obtained with two mr. s^{-1} incident on the target, which is comprised of a heavy metal layer in intimate contact with a secondary electron enhancing storage layer.

Most imaging systems used in the application of isotopes form an image in a large area scintillation counter crystal, the light output of which is detected by photo-multiplier tubes for storage, subsequent processing and image formation[1]. The work reported here has as its goal the combination of the above image forming capabilities into one relatively simple image transducing surface that would permit variable storage rate displays utilising television techniques.

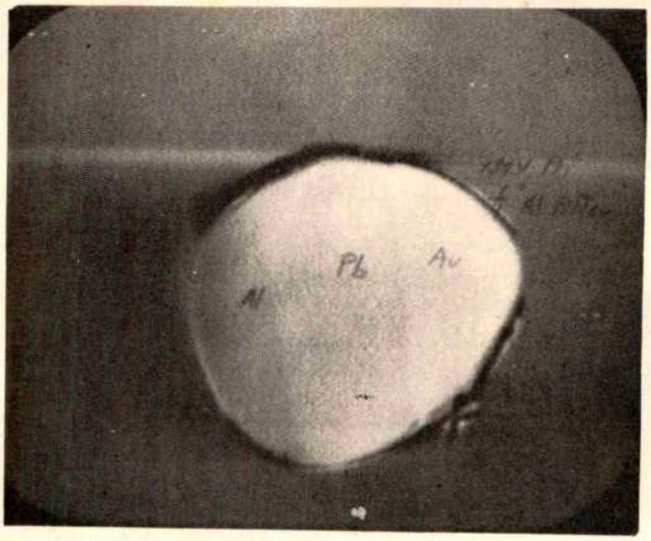

Fig. 1 Variation in sensitivity of metallonicon with differing metal converting layers. The Pb and Au layers are both 6 μm thick. Al is the uncoated signal plate. Note the improved performance of the system obtained when using the Au layer in combination with the CsI storage and signal enhancing layer. The X-ray generator used produces 75 kV potential self-rectified with 130 V applied to the primary. X-ray tube current was 5×10^{-4} A. Tube to target distance was 300 mm. Filter in beam 8 mm aluminium. Target diameter 100 mm. Image display rate 30 frames per second.

At penetrating-radiation energy levels exceeding 150 kV potential, heavy metal intensifying screens are used in preference to, or in combination with, fluorescent screens to provide increased speed and detail resolution for the radiographic process[2,3]. Attempts have been made in the past to utilise this image forming technique in the construction of penetrating-radiation image converters[4]. These have, in general, not been of great practical consequence because of the extremely

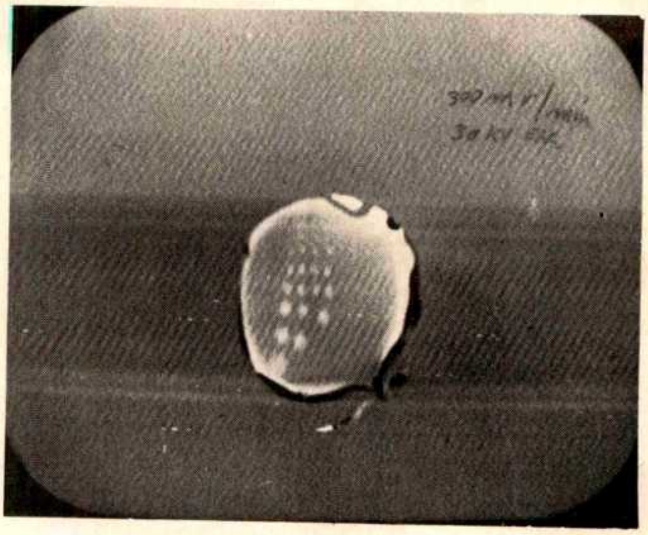

Fig. 2 Resolution obtainable at measure dose rate of 5 mr. s^{-1} at face plate of tube. Object is Pb sheet with holes ranging in size from 5 mm at bottom of image to 1 mm at top. Magnification of object detail due to X-ray generator–object–image target geometry. Diameter of image target 100 mm. X-ray generator 60 kV potential self-rectified. X-ray tube current 2×10^{-3} A. 25.4 mm aluminium placed between object and image tube target to achieve level and quality of radiation noted. X-ray tube to image target distance 300 mm. Image display rate 30 frames per second.

low yield of the absorbed quanta to photoelectron process and the relatively short image integration time

Secondary electron enhancing materials such as KCl and CsI are routinely used as the image storage layer in television camera tubes capable of operation at the low light levels encountered in astronomical studies[5] The tube reported here combines on one scanned surface the high energy conversion method of radiography with the storage and image enhancing features of the SEC target Even at its present stage of development it exhibits characteristics that indicate it has potential value in many applications concerned with the imaging of high energy penetrating radiation fields

This new image tube combines a heavy metal conversion layer (Au, Pb, or Pt) with a high resistivity secondary electron enhancing layer (CsI) to form the target of a television camera storage tube The target of the tubes constructed so far is made using an aluminium substrate covered with an evaporated layer of gold about 6 μm thick upon which a layer of low density CsI is deposited The low density CsI layer, prepared by evaporating CsI in an argon atmosphere of approximately one torr, has a mass per unit area of roughly 4 5 g cm^{-2} When a field is established between the aluminium signal plate and the back surface of this layer by the scanning process, secondary emission is enhanced due to pores in the CsI layer The electron gain obtained with these low density layers is higher by some 40 times than those obtained using bulk CsI

Our work indicates that it is necessary to have a particular heavy metal emitting layer produce the photoelectrons An experiment in which the heavy metal layer was both eliminated and varied in composition is illustrated by Fig 1 Comparison studies between equal thickness Pb, Pt, and Au layers indicate that the Au layer is preferred in combination with the CsI film

Tubes fabricated with 100 mm diameter Au–Cs targets have been tested utilising a half-wave rectified X-ray generator whose effective radiation energy was measured to be 35 kV potential Response time of the tube when scanned at a rate of 30 frames per second was such that a moving object exhibited strobing due to the pulsating nature of the X-ray source In other experiments, the scanning beam was used to charge the target to approximately 40 V potential and then turned off The target was then exposed to 1 0 mr of radiation during a 1 s exposure Following a wait of 5 min, the scanning beam was turned on, at which time the image resulting from this 1 s exposure was seen as the charge pattern was equalised by the scanning beam Figure 2 illustrates the images obtainable at incident dose rates of 5 0 mr s^{-1} of radiation of 30 kV potential effective under continuous scanning at 30 frames per second

The effective X-ray energy of 35 kV potential implies that the photoelectrons were ejected from the L-shell in Au Continuing work indicates that by utilising the high energy photoelectrons and secondary radiation quanta from the K-shell in the energy range of 150 keV together with a micro-pyramidal substrate, a tenfold increase in sensitivity is obtainable

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Monomolecular treatment of chemical oscillation

THE mechanism of the Belousov oscillating reaction—the catalytic oxidation of malonic acid with bromate—is highly sophisticated Although considerable progress has been made in revealing the kinetics and mechanism of both the complete oscillating system[1,2], and some of the composite reactions (especially those occurring between bromate and catalyst[3,4]) many points need further investigation

Looking at the temperature dependence of chemical oscillation and the role of different catalysts we thought to propose a simple treatment for the Belousov oscillating reaction The basis for this lies in the fact—supported by early observations, and by our calorimetric and polarographic results—that the most important reaction in a Belousov oscillating system is the autocatalytic oxidation of the catalyst (M^{n+}) by bromate in the presence of malonic acid by the formation bromomalonic acid (BrMA),

$$BrO_3^- + 4M^{n+} + CH_2(COOH)_2 + 5H^+ \longrightarrow BrCH(COOH)_2 + 4M^{(n+1)+} + 3H_2O \qquad (1)$$

In this phase of the oscillating reaction, the rate of heat evolution is very high, and this is synchronised with a sudden potential jump and decrease in bromide concentration[5] This phase is followed by one where ordinary redox reactions (such as the reduction of $M^{(n+1)+}$ by malonic acid, bromate–bromide reaction) proceed and the rate of heat evolution is low The latter phase can be regarded as a comparatively 'restful' one When the bromide concentration in the system falls below a critical value (~3 10^{-6}M) (ref 1), the autocatalytic reaction is switched on again These processes follow each other periodically Thus, the Belousov oscillating reaction can be regarded as a series of autocatalytic reaction 'bursts' occurring with a certain frequency

This consideration led us to treat chemical oscillation as a 'monomolecular' reaction As a first order rate constant (k_ω) we shall use the reciprocal of the period time at initial concentrations From k_ω values measured at different temperatures using the Arrhenius equation the 'activation energy' of chemical oscillation (E_ω) can be calculated (ω refers to chemical oscillation expressing its periodic character)

Reacting oscillating systems with three different catalysts (Ce^{3+}, Mn^{2+} and $Ru(dipy)_3^{+2}$ were investigated at 15, 25 and 35° C The oscillating systems contained malonic acid (0 4 m), potassium bromate (0 1 M), sulphuric acid (1 M) and a catalyst (0 0005 M) (system 1) From the frequency of the second (or third) period of chemical oscillation the activation energy of the oscillating reactions was calculated The frequency changes with time because of concentration changes in the reactants, so it is necessary to choose an early period Figure 1 shows the log k_ω against $1/T$ plots From Fig 1 it is obvious that the Arrhenius equation is valid in the temperature range investigated and that E_ω is practically identical with all the three systems E_ω = 16 1 ± 0 2 kcalorie mol^{-1} (67 5 ± 0 8 kJ mol^{-1})

Using the absolute rate equation, the 'entropy of activation' of the oscillating reactions (ΔS_ω*) was also calculated Actually, ΔS_ω* is the probability of 'autocatalytic reaction bursts' Its value is −12 5 calorie mol^{-1} deg^{-1} (−52 5 J mol^{-1} deg^{-1}) with all three catalysts

We conclude that neither E_ω nor ΔS_ω* depends on the nature (for example, the redox potential) of the catalyst This identi[illegible] the chemistry of the 'burst' reaction (1)

Oscillating systems with different initial concentrations were also investigated In a chemical system containing malonic acid (0 4 M), potassium bromate (0 1 M), sulphuric acid (0 5 M) and cerium (III) or manganese (II) (0 005 M) system 2 (16 5 ± 0 2 kcalorie mol^{-1}) 69 1 ± 0 8 kJ mol^{-1} was obtained for E_ω which is in a rather good agreement with the value obtained for system 1 k_ω, and thus the ΔS_ω*, values, however, differ

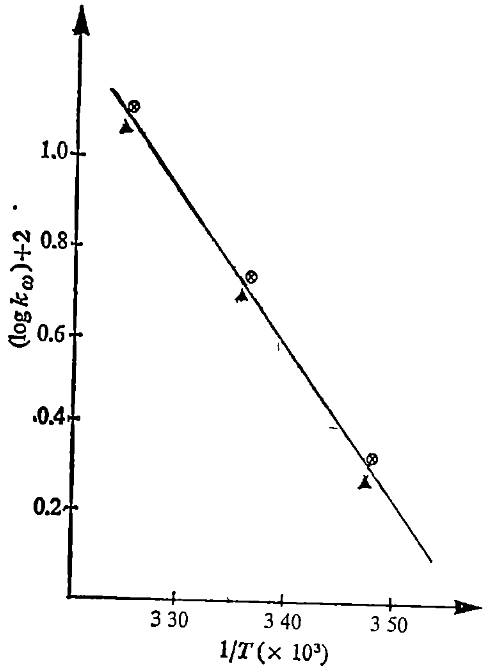

Fig 1 Plot of the logarithm of the reciprocal of the period time ($\log k_\omega$) against $1/T$ ($T=288{,}298$ and 308 K) The chemical systems containing malonic acid (0 4 M), potassium bromate (0 1 M), sulphuric acid (1 M) and catalyst (0 0005 M) ○, Ce^{3+}, × Mn^{2+}, ▲, Ru $(dipy)_3^{+2}$

with the two systems (In system 1 $k_\omega = 0\ 053$ and in system 2 $k_\omega = 0\ 015$ at 25° C) E_ω—at least within certain concentration ranges—is independent of the composition of the oscillating system the probability of 'bursts', however, is a function of the composition

Are there any characteristics of the oscillating reactions which manifest themselves when one catalyst is replaced by another? An exhaustive answer cannot be given yet

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Memory for words

LISTENERS do not ordinarily retain the syntax of a sentence for longer than is necessary to grasp its meaning, they rapidly forget both superficial 'surface structure' and underlying 'deep structure'[1-3] What they retain is the sense of the sentence, evidently divorced from its syntax It has been argued that this sense might consist of an associative structure linking representations of the words in the sentence [illegible] meaning is divorced from syntax, it might also be divorced from words Indeed, we shall argue that a semantic representation need not incorporate any direct information about the lexical categories of words, it merely appears to do so because they can often be reconstructed from the meaning of a sentence It follows, of course, that the specific wording of a sentence should ordinarily be rapidly forgotten[5] One simple test of this hypothesis, avoiding the predictability of words from meaning, is to examine a person's ability to recollect whether a certain element of meaning was conveyed by a noun or a verb (Does the reader recollect with any confidence whether, for example, we wrote earlier, "the divorce of meaning from syntax", or "meaning is divorced from syntax"?) We predicted that information about lexical categories would tend to be forgotten if subjects were unaware that their memory for a sentence was to be tested, but that it would tend to be remembered if subjects expected their memory to be tested

Two independent groups of subjects were told that their task was to make up two sentences continuing the theme of a short spoken passage The members of the 'memory' group (M) were also told that they would be given a memory test for the passage, the members of the 'incidental' group (I) were given no such instruction After listening to the tape recorded passage, which described a brief incident in a science-fiction story, both groups were given recognition tests for two of the sentences in the passage

To test the hypothesis, we obviously had to use sentences whose meanings do not allow the lexical categories of their constituent words to be easily reconstructed The third sentence of the spoken passage was therefore one of the following items

(1) The owner of the magic staff dispatched the ship
(2) The dispatcher of the ship owned the magic staff
(3) The owner of the ship dispatched the magic staff
(4) The dispatcher of the magic staff owned the ship

The first pair of sentences are very similar in meaning—what one presupposes the other asserts—yet they convey their meanings by way of totally dissimilar lexical categories, for example, the information conveyed by the verb 'dispatched' in sentence (1) is conveyed by the noun 'dispatcher' in sentence (2) The second pair of sentences stand in a similar relation to one another, but their meanings are quite different to the meanings of the first pair of sentences in the first pair it is the ship that is dispatched, in the second pair it is the magic staff that is dispatched The fifth and final sentence of the spoken passage was selected from the following set of sentences constructed according to the same principles

(5) On earth, the interrogator of the Martians advised the President
(6) On earth, the adviser of the President interrogated the Martians
(7) On earth, the interrogator of the President advised the Martians
(8) On earth, the adviser of the Martians interrogated the President

The sense of the remaining sentences in the passage was sufficiently flexible to accommodate any combination of the different versions of the third and fifth sentences Thus, four different variations of the passage were recorded involving the following combinations of sentences (1) and (6), (2) and (8), (3) and (7), and (4) and (5)

After listening to the passage, each subject was given a recognition test for the third sentence followed by a recognition test for the fifth sentence These tests involved presenting the four versions of each sentence, together with their equivalents in the passive voice, both of the two sets of eight sentences were presented in different randomised orders The subjects, 16 students at Sussex University, were assigned in rotation to a group and to one of the four passages They were tested individually, and they were allowed, if necessary, to make three attempts in each test to select the actual sentence that had occurred in the passage

There was no reliable difference between the groups in their ability to retain the correct meanings of the sentences two subjects out of eight made semantic errors in group M, and four subjects out of eight made semantic errors in group I The crucial distinction between the groups concerned those syntactic errors that reveal an inability to remember the lexical categories of the original sentence even though its correct meaning has been retained, for example, the selection of sentence (2) where the original was, in fact, sentence (1) Only one such error was made by a subject in group M, whereas six subjects produced a total of eleven such errors in group I, the difference in the number of subjects making errors in the two groups was reliable ($P=0$ 02, Fisher Yates exact test) When a subject makes two semantic errors his other attempt is likely to be a guess even if it is correct in meaning, so we excluded the data of two such trials from the totals although their inclusion does not alter the basic pattern of the results

A second experiment with a similar design but different materials examined the ability of a further twenty-four subjects to remember such sentences as

(9) The borrower of the money purchased the car and the seller of the fridge bought the lawn-mower
(10) The purchaser of the car borrowed the money and the buyer of the lawn-mower sold the fridge

This task proved to be more difficult than remembering the sentences of the first experiment The subjects in group I made a total of twenty-two semantic errors and the subjects in group M made a total of eight semantic errors, and the difference between the groups was reliable ($S_0=$ 73 25, $P<0$ 015, Mann-Whitney test) Yet every subject made at least one error symptomatic of a failure to retain lexical categories, for example, confusing sentence (9) with sentence (10)

The results of the experiment suggest that even when listeners successfully recall the meaning of a sentence they can only remember whether an element of this meaning was conveyed by a noun or a verb if they know that they are to receive a memory test They are then liable to implement a method for retaining verbatim information Otherwise, lexical categories appear to be rapidly forgotten along with the superficial and underlying syntax of the sentence One could defend the thesis that sentences are remembered by forming an associative structure linking representations of their nouns and verbs by arguing that the associative machinery deals with nominals derived from verbs in a very similar way to verbs themselves But this argument runs the risk of rendering the thesis untestable It is precisely such items that prevent the automatic reconstruction of lexical categories from the meaning of a sentence One could also defend the thesis that at least word stems are remembered, since it was only the suffixes of words which were manipulated (together with the syntax of the sentence) But this view is incompatible with the results of previous experiments[2 3] We prefer to think that meaning is something that can be put into words with a suitable syntactic structure rather than a direct representation of the structured set of words itself The words and their structure can be thrown away as soon as their meaning has been grasped If this thesis is correct, it is misleading to talk of a memory for the nouns and verbs of a sentence unless one is concerned with a deliberate attempt to memorise them It is similarly misleading to talk of a memory for the underlying subject and object of a sentence, or for its underlying cases Such terminology is tied to the words that occur in a sentence and, as we have shown, it can be misleading to talk of a memory for words

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Genotype interaction with undernutrition and external environment in early life

IN spite of the well documented effect of early undernutrition on body and brain size in adult animals[1-3], its reported influence on behaviour has been less consistent Changes in performance in learning tasks and activity tests have been found following undernutrition in early life[4-7] Several studies, however, have shown little or no effect of such a manipulation[8-10] Here we consider two factors which may contribute to variability in later behavioural response to early life undernutrition

Recent work indicates that the type of environment in which animals are reared may modify the behavioural consequences of undernutrition[11-13] Subtle changes in rearing conditions in different laboratories might account for the apparent lack of effect of undernutrition in some cases and its significant influence in others Another important variable which could account for variability in results is the genetic background upon which nutritional treatments are imposed Thus, the effects of undernutrition during the period of rapid development may differ according to the particular genotype selected for study

We examined the interaction of genetic background with both early life undernutrition and differential environmental conditions The C57BL/6J and DBA/2J mouse strains were selected for our experiments because they differ genetically in the control of a large number of behavioural, biochemical and physiological characteristics[14]

Mice were raised in three different environments and two levels of dietary protein Five males and five females from each mouse strain were exposed to each of the above conditions

Litters from dams of the C57BL/6J and DBA/2J mouse strains which had been previously mated in our laboratory were reared after parturition in *ad libitum* or undernourished nutritional conditions in either enriched normal or impoverished environments The enriched condition consisted of a large, clear, lucite box (75×40×20 cm high) divided diagonally in two, containing various manipulanda, the normal condition was represented by opaque, plastic home-cages (30×13×12 cm), the impoverished group was reared in sound-deadened, ventilated chambers in normal home-cages but with sensory input restricted by covering the top of the cages with a white painted, fitted metal sheet Control or undernourished animals were fed a 27% or 8% casein diet respectively, containing 10% fat with minerals and vitamins added The diets were made isocaloric by the addition of dextrose and contained 0 2% saccharin to improve palatability The litters were culled to four animals on day 4 of life After removal of the mother at 21 d, the weanlings remained in the appropriate environmental and nutritional treatment groups until 42 d after birth At this age, they were separated by sex and maintained in normal laboratory conditions with food and water available *ad libitum* until behavioural tests were carried out 90–124 d of age

At this time, animals were exposed to an illuminated (30 foot candles) Lehigh Valley photactometer, 45 cm in diameter, with a solid wood floor painted white, for 2 min on three consecutive days The frequency with which animals intersected the light sources activating the photocells was recorded automatically every 30 s Rearing and defaecation frequency were scored by an observer for each daily trial Two days after the final open-field test, animals were exposed to a 'novel-object test in the same apparatus The number of visits to,

Table 1 The results of open-field and 'novel object' tests administered to mice of the C57BL/6J and DBA/2J strains 90–124 d of age fed either an 8 or 27% casein diet for the first 42 d of life

Test	C57BL/6J Normal ($n = 25$)	C57BL/6J Undernourished ($n = 28$)	DBA/2J Normal ($n = 30$)	DBA/2J Undernourished ($n = 28$)
Mean daily open-field activity (counts per 30 s period)	19 86	16 19	23 26	23 24
Mean daily 'novel object' defaecation frequency	1 93	2 70	4 03	3 53
Mean daily open-field rearing frequency	2 94	2 01	6 87	6 24

Means for replacing missing data were utilised in seven cases in the C57BL/6J plus two cases in the DBA/2J strain The effect of these data substitutions were taken into account in the calculating degrees of freedom, error terms and F ratios Separate analyses of variance of each measure were carried out within each mouse strain In the C57BL/6J strain, undernourishment reduced activity ($F = 3\ 90$, d f 1, 51, $P<0\ 06$) and rearing frequency ($F = 4\ 05$, d f 1, 48, $P<0\ 05$) in the open-field test In the 'novel object' test, undernourishment significantly increased defaecation scores ($F = 4\ 19$, d f 1, 49, $P<0\ 05$) Early life undernutrition had no overall effect in the DBA/2J strain

and the time spent in the centre square containing a black spring mounted on a lucite base were recorded for a single 5 min test session Total activity and number of defaecations were also recorded

Analysis of variance was used to evaluate the main effect and interaction of early life undernutrition and environment and sex within each mouse strain Where measures were repeated either within daily sessions or on consecutive days on the same animals, appropriate partitions of variance were also made[15] This statistical treatment was applied to the three open-field test measures and the four 'novel-object' test scores[15]. Rearing and defaecation scores were transformed by a $\sqrt{(x+0\ 5)}$ transformation to normalise their distribution

Females defaecated significantly more than males in the open-field test in both strains ($F = 11\ 17$, d f 1, 48, $P<0\ 001$ in C57BL/6J strain $F = 5\ 18$, d f 1, 46, $P<0\ 05$ in the DBA/2J strain), but on other behavioural measures, no sex differences were found Higher-order interactions involving sex were not consistent, easy to understand or significant at a very high level and, for this reason, data from both sexes are considered together

In the C57BL/6J strain, early life undernutrition significantly reduced rearing frequency and tended to reduce activity, although the probability level associated with this effect was not very satisfactory ($P<0\ 06$) It also increased defaecation scores in the 'novel-object' test In contrast, the statistical analysis revealed no overall effect of nutritional restriction on any of the seven measures derived from the two tests in the DBA/2J strain Table 1 shows the effects of early life undernutrition in the two strains on those measures which discriminated undernourished from control groups in the C57BL/6J strain

In contrast to the effects of dietary restriction, the external environment for the first 42 d of life substantially influenced mice of the DBA/2J strain On five out of seven behavioural measures, a significant effect of environmental manipulaton was observed in the DBA/2J strain Environmental impoverishment reduced open-field activity and rearing frequency It also reduced activity levels and visits to the centre square and increased defaecation scores in the 'novel object' test

By comparison, the only statistically significant effect of early life environment in the C57BL/6J strain was in the 'novel object' test where impoverishment reduced the frequency of visits to the centre square Table 2 depicts the effects of the manipulation of environmental conditions in early life in the two strains Although the behavioural responses of the 2 strains to early nutritional restriction differed, effects on body weight at the time of testing were almost identical Undernourished animals were 77% and 78% ($P<0\ 001$) of control animal weights in the two strains

Although no effect of undernutrition was detected in the DBA/2J strain when all experimental conditions were combined, undernutrition did consistently affect behaviour of DBA/2J mice raised in the normal environment The interaction of environment × diet × time for open-field activity was statistically significant ($F = 2\ 938$, d f 6, 136, $P<0\ 025$) in the DBA/2J strain Undernutrition was correlated with reduced activity in DBA/2J mice raised in the normal environment, the effect being restricted to the first minute of the 2 min test In contrast, there was no consistent effect of undernutrition in DBA/2J mice raised in the enriched or impoverished environments on this measure A second statistically significant interaction of environment with diet ($F = 3\ 8$, d f 2, 46, $P<0\ 05$) indicated that, if DBA/2J mice were raised in the normal environment, undernourished animals defaecated more than controls in the 'novel object' test By comparison, nutritionally deprived animals raised in the enriched or impoverished environments defaecated less than controls Therefore, although undernutrition had a greater effect in the C57BL/6J strain, the occurrence of these interactions in the DBA/2J strain emphasises the relative nature of the dependence of nutritional and environmental influences on genotype

Our results which demonstrate genetic influences on behavioural response to both early life undernutrition and environmental manipulations should be considered in the light of previous discussions of gene–environment interactions in these mouse strains[16–18] Furthermore, the fact that genetic factors can influence an organism's response to undernutrition suggests a possible explanation of why undernutrition has been shown to influence some human groups but appears to leave others unaffected[10]

Table 2 The results of open-field and 'novel object' tests administered to mice of the C57BL/6J and DBA/2J strains 90–124 d of age raised in environmental enrichment, normal home-cage conditions or environmental impoverishment

Test	C57BL/6J			DBA/2J		
	Enriched ($n = 14$)	Normal ($n = 20$)	Impoverished ($n = 19$)	Enriched ($n = 19$)	Normal ($n = 20$)	Impoverished ($n = 19$)
Mean daily open-field activity per 30 s	18 40	17 72	17 95	25 05	24 76	19 93
Mean daily open-field rearing frequency	2 47	2 38	2 58	8 97	6 82	3 88
Mean novel-object activity scores ,	148 6	131 96	121 85	273 85	202 05	143 40
Mean novel-object defecation frequency	2 05	2 40	2 50	3 10	3 45	4 80
Mean daily novel-object visits	0 88	0 61	0 39	1 28	0 75	0 35

Impoverishment reduced activity ($F = 3\ 53$, d f 2,46, $P<0\ 05$) and rearing frequency ($F = 6\ 73$, d f 2,46, $P<0\ 01$) in the open-field test in the DBA/2J strain In the 'novel object' test, impoverishment decreased activity ($F = 7\ 25$, d f 2,46, $P<0\ 005$) and visits to the centre square ($F = 3\ 52$, d f 2,46, $P<0\ 05$) while increasing defaecation scores in the same situation ($F = 4\ 6$, d f 1,46, $P<0\ 025$) in this strain In the C57BL/6J strain the only statistically significant effect of environmental manipulation was visits to the centre square in the 'novel object' test, where impoverishment reduced the frequency of visits ($F = 3\ 58$, d f 2,50, $P<0\ 05$)

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The earliest conifer

SMALL leaves and a leafy shoot from the Low Westphalian B (Upper Carboniferous) near Wakefield, Yorkshire, represent the oldest direct evidence for the existence of the order Coniferales (conifers) yet discovered. This material is preserved both as compression fossils and as charcoal.

Unlike most other fossil plants from the Coal Measures these leaves show both epidermal characters and internal structure. Most of the leaves are small (1–5 mm long), narrowly triangular (Fig. 1*a*) and are occasionally forked (Fig. 1*b*), similar in general appearance to Florin's genus *Lebachia*[1]. The leaves are hypostomatic and have a denticulate margin (Fig. 2). The lower epidermis has two wide stomatal bands with closely spaced stomata which often share subsidiary cells and are monocyclic. The leaves are uninerved and the non-stomatal areas of both the lower epidermis and all of the upper epidermis consist of elongate cells interspersed with smaller cells. The outer (periclinal) wall of the epidermal cells (presumably including both cuticle and underlying cellulosic wall) is very thick (Fig. 3); this seems to have been burnt off in some of the leaves. A layer of fibrous tissue immediately underlies the epidermis, while mesophyll tissue is generally less well preserved. The nature of the vascular trace is ambiguous but it is believed that the tracheids have annular or scalariform thickenings.

This earliest record of conifers has both evolutionary and environmental significance. It is suggested that the leaves have been charcoalified by forest fire and have been fragmented during transport to their site of deposition. Many of the leaves show typical features of rapid burning such as collapse of epidermal cells (Figs 2 and 4), occasional absence of cuticle and the three-dimensional nature of the leaf—the cell lumina still remaining open (uncrushed; Fig. 3). The early conifers are rare, previous records coming from the Westphalian D (high Upper Carboniferous) and above. These all belong to the broadly based form genus *Walchia* Sternberg which Florin subdivided into two genera, *Lebachia* and *Ernestiodendron* on differences in their leaf arrangement, reproductive structures and cuticular characters. This new material has not been assigned to a genus as yet but is believed, from the information available, to belong to the family Lebachiaceae. No reproductive structures have yet been found but it is believed that the information obtained from the vegetative material is sufficient to justify inclusion in both the order Coniferales and the family Lebachiaceae. This will be discussed in detail elsewhere.

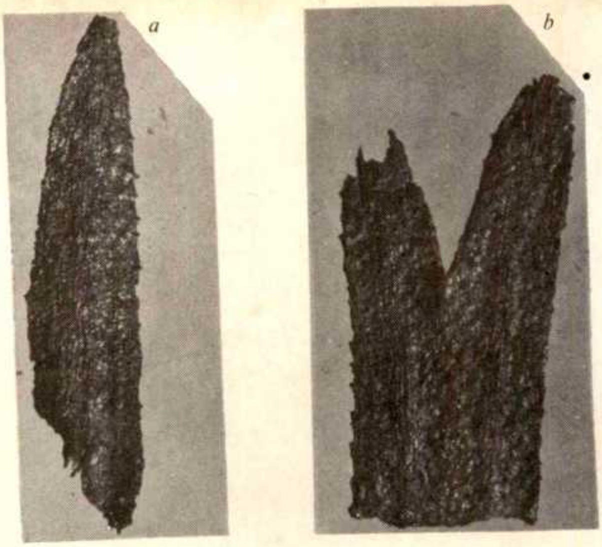

Fig. 1 Light micrographs of charcoalified conifer leaves. *a*, Small needle-like leaf (×30); *b*, forked leaf (×30).

Such early conifers have not been found commonly in the fossil record presumably because their environment of growth differs from those plants normally found in the Coal Measures[2]. The depositional environments in the Stephanian (high Upper Carboniferous) and Permian, especially in Europe, favoured the preservation of such plants.

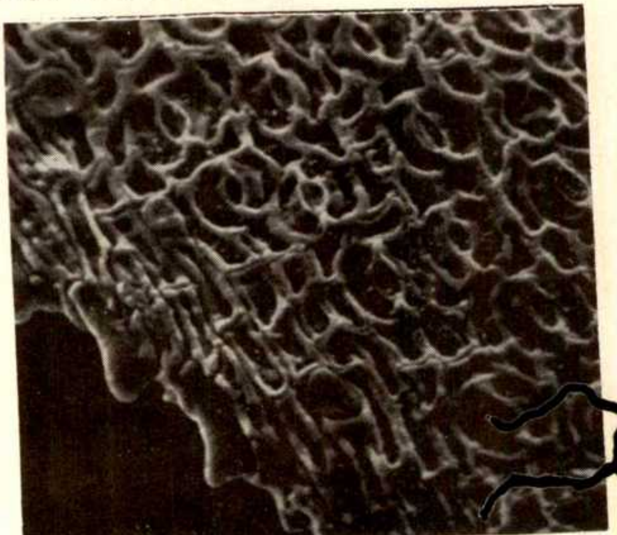

Fig. 2 Scanning electron micrograph of part of the lower epidermal surface of a leaf showing a stomatal band and a denticulate margin (×465).

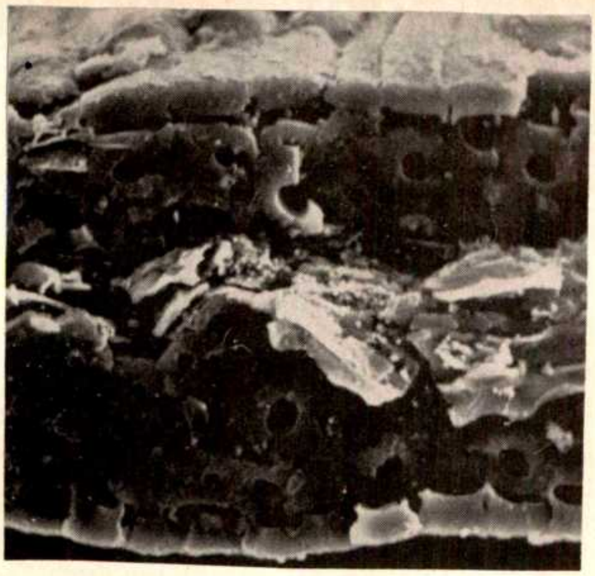

Fig. 3 Scanning electron micrograph showing part of the cross section of a typical leaf (thick cuticle, subepidermal fibres and crushed mesophyll tissue) (×900).

Pollen called *Potonieisporites* Bharadwaj has been shown to belong to the genus *Lebachia* Florin in the Permian[3]. Pollen of this type is also known from the Upper Carboniferous (Westphalian and Stephanian). It is now evident that such pollen may have been produced by conifers.

The discovery of conifer material, by bulk maceration of thin clay bands containing fusain within a poor coal, is important to the understanding of Upper Carboniferous floras as a whole. There is growing evidence that a fairly substantial flora is present in a charcoalified state, possibly of plants living associated with the conifers, and this may give insight into the palaeoecology of these (?hinterland) floras.

Harris[4] has discussed forest fire in the Mesozoic and others (A.S., and de Klerk, unpublished) have considered it as a fossilising mechanism in the Tertiary. It is not surprising, therefore, that forest fire may have played an important role in the Upper Carboniferous and the nature of fusain bands within coals and shales must again be open for debate. Early records of conifers in the form of such charcoalified fragments may now be sought using the bulk maceration technique in the hope of learning more of their origins and ecology.

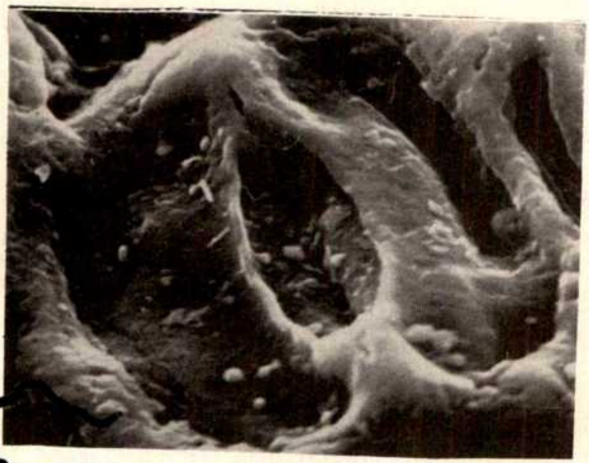

Fig. 4 Scanning electron micrograph of a single stoma showing upstanding anticlinal walls and collapsed guard cell walls. (An alternative explanation is that the guard cells are sunken below the surface and exposed only around the stomatal pit at the centre and that there are only two subsidiary cells which have themselves collapsed.) (×4,500).

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A model for stably inherited environmentally induced changes in plants

THE phenotypes of some inbred lines of *Linum usitatissimum* (flax) and *Nicotiana rustica* can be altered environmentally by fertiliser treatments, and the fertiliser-induced phenotypes show stable inheritance independent of the inducing conditions[1–3]. In flax, the stably inherited large (L) and small (S) phenotypes can be induced from the plastic (*Pl*) genotype *Stormont Cirrus* by one generation of growth with high nitrogen or phosphorus fertiliser treatments respectively. The induced phenotypes of L and S, differing for example in plant weight and degree of branching, behave in most respects as distinct genotypes and have been termed genotrophs.

Current genetic theory offers at least two explanations for such changes; biological examples of both types are examined in relation to the flax system.

(1) Informational nucleic acid may be introduced into flax under the inducing treatments, and be maintained in subsequent generations. Several groups[4–7] have shown that exogenous DNA can be taken up, expressed and maintained by plants, though whether it is integrated into the host genome is uncertain. In flax, possible nucleic acid donors could be among the soil flora. Under the inducing treatment the soil flora may change, or conditions for uptake or incorporation of nucleic acid may be favoured. It could be established if flax DNA contains sequences hybridisable to DNA extracted from soil flora. This idea predicts that the induced changes should not occur with flax grown under sterile conditions.

Changes in the DNA content of the flax genotrophs do occur[8,9], but their relevance remains uncertain because the changes can be reversed without loss of either the induced phenotype or its stable inheritance[10]. The DNA changes are partly caused by variation in the redundancy of ribosomal DNA[11]. It is unlikely that this change would be the primary event in genotroph induction, but could be a consequence of a regulatory switch such as described below.

(2) Some change in a regulatory circuit has been effected by the inducing conditions; this change persists after removal of these conditions, and involves no qualitative change in genome. Lysogens of bacteriophage λ show a temperature-induced loss of immunity in the host *Escherichia coli*, that is stably inherited independent of the inducing conditions with no change in the phage genome[12,13]. λ prophage synthesises the *cI* gene product, the repressor protein, that renders the host *E. coli* immune to further homoimmune λ infection. *cI*857 prophage has a temperature-

sensitive repressor protein active at 32° C but inactive at 42° C *E coli* containing prophage with genotype *c*I857 N^- (O^- or P^-) are immune at 32° C, but growth at 42° C inactivates the repressor and immunity is lost At 42° C synthesis of the *cro* gene product (normally repressed by repressor) occurs and *cro* product prevents further repressor synthesis On return to 32° C, *cro* product continues to prevent repressor synthesis, immunity is not regained and the immunity loss is stably inherited and dependent upon formation of the active *cro* product

The λ system is analogous to genotroph induction and suggests a model for the induction and stable inheritance of the *L* and *S* genotrophs from the *Pl* flax genotype Figure 1*a* represents the regulatory system of the *Pl* phenotype under non-inducing conditions, this system contains three regulatory proteins which act and remain in the flax cell nuclei and are coded on distinct structural genes A control protein (CP) is made which controls its own synthesis in a positive manner CP is also a negative control element preventing synthesis of two other regulatory proteins, an activator protein (AP) and an inhibitor protein (IP) AP and IP are involved in triggering sequences of events leading to the L and S phenotypes The model is not dependent upon the precise mechanism of these events, though modulation of a plant hormone level may be involved The site of CP regulation is shown as transcriptional, but could be translational Figure 1*b* illustrates the switch from the *Pl* to the L form under inducing conditions High nitrogen fertiliser treatment increases the concentration in the plant of some effector molecule E^L

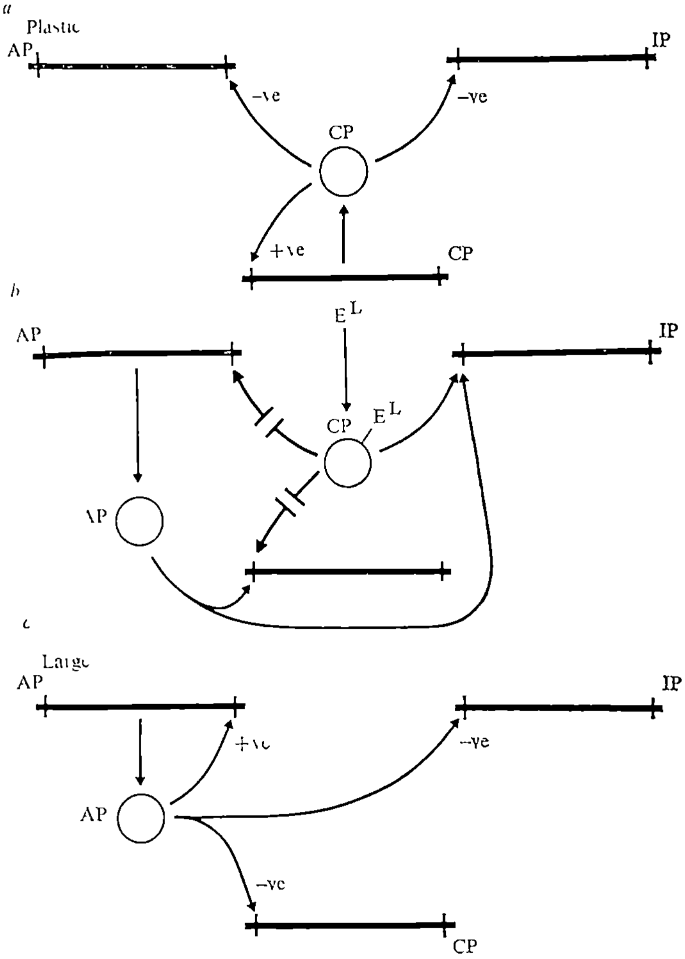

Fig 1 Regulation of the *Pl* phenotype and the induction and stable inheritance of the L phenotype in flax The structural genes for the three regulating proteins are indicated by heavy lines, for simplicity the haploid situation is shown, the inbred lines being assumed isogenic The regulating proteins are shown as circles and behave as transcriptional control elements

CP binds E^L to form a CPE^L complex inactive in controlling CP and AP synthesis, but still active in preventing IP synthesis Consequently AP synthesis starts, CP synthesis stops and IP synthesis remains inhibited AP triggers the L phenotype event sequence, inhibits CP and IP synthesis, and is a positive control element in its own synthesis AP will now continue to be made in the absence of the inducing conditions (Fig 1*c*), and the L phenotype will be stably inherited A similar regulatory switch triggers the S phenotype from the *Pl* one under inducing conditions Here some effector E^S increases in concentration in the plant inactivating CP so that IP is synthesised which triggers the S phenotype event sequence IP has analogous control properties to AP and the S phenotype is stably inherited

The model explains other findings in the flax system The F_1 progeny of an $L \times S$ genotroph cross are intermediate in plant weight, show high plant weight variance and are non-inducible by fertiliser treatments[1] In such a cross both zygotes contribute equal amounts of the nuclear regulatory proteins AP and IP, there are no maternal effects, and an unstable mix of both regulatory proteins each capable of inhibiting synthesis of the other occurs In phage λ, the outcome of such a match between opposing gene functions can depend upon additional variables[14,15] In flax, individual plants should have a certain probability of changing from the mixed to the AP or IP regulatory mode This could result in a wide population variance in plant weight, the extremes of which (sole AP or IP control) would be stably inherited Similar explanations exist for the outcome of crosses that mix the CP and AP or IP regulatory proteins

The primary sites of action of the AP and IP proteins could be modulation of plant hormone levels or regulation of synthesis of growth inhibitors Determining the levels of plant hormones and growth inhibitors in *Pl*, L and S plants seems a necessary step to biochemical analysis of induced changes

The model predicts the properties of some mutations in the regulatory genes A search for and genetic analysis of such mutants will both test the model and give more information on the nature of induced changes Three pleiotropic loci, the structural genes for CP, AP and IP, should be identifiable and distinguishable from mutations in other genes altering components not directly related to the regulatory switch Mutants in these loci should show stable L or S phenotypes independent of inducing conditions, or plastic phenotypes with altered inducibility

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Control of food intake by energy supply

A WIDE variety of metabolic influences on appetite have been proposed, including glucose utilisation rate[1], influences from adipose tissue[2,3] or plasma amino acid pattern[4,5] and the heat increment during absorption of a meal[6] It is difficult to relate short term effects of the metabolism of absorbed nutrients which indeed occur[7], to the long term precision with which energy exchange is often regulated

A direct signal from fat stores to the brain has been postulated[3] but an alternative is the modulation of a short term 'glucostatic' signal by a 'lipostatic' signal[8] A more general proposal along the latter lines is that the signal at the neural receptors controlling feeding in the omnivore reflects the current supply of energy for intermediary metabolism from whatever source, including sugars, amino acids, fatty acids, ketones, glycerol and lactate[7,9] There is some reason to believe that this metabolic control is primary in the control of food intake, with control by sensory and gastrointestinal mechanisms normally being adjusted according to metabolism[11–13]

The theory that current energy supply is the primary factor controlling appetite has now been incorporated for the rat in a computerised physiological control model illustrated in block diagram form in Fig 1 The pattern of feeding is correctly predicted within the limits of accuracy of the physiological data which completely determine the computation (Table 1)

The rate at which the rat feeds is taken to be constant throughout a meal, as an initial simplification A feeding rate of 1,000 calorie min^{-1} (about 0 3 g min^{-1}) is an intermediate value from the literature[14,15] The rate at which the gut clears nutrient energy is taken to be proportional to the square root of the amount of energy contained in the gut, a function based on studies of gastric carbohydrate clearances[9,16] The constant of proportionality (K) is higher (0 9) by night than by day (0 6) in the rat, a nocturnal animal, this value is taken to change linearly over 2 h around dawn and dusk These calculations predict the variations in rate of absorption of energy-yielding substances during and between meals

Another source of variation in total energy flow is storage and mobilisation of fat This has been based in the model on the results of Le Magnen and Devos[17], which indicate mainly net subtraction of energy from fat store by day and net addition of fat by night

Threshold values for initiation and termination of feeding purely on the basis of total energy flow were based on amounts found in the gut at the start and end of meals and on the effects of infusing glucose into the hepatic portal vein in the rat[18] Feeding is initiated when energy supply rate falls to below 18 calorie min^{-1} It is fully suppressed on a single diet when energy is supplied at or above 60 calorie min^{-1} In this initial approximate model these estimated values are taken to be without statistical variation

The complete system was programmed for a digital computer, using a method of successive addition all variables were adjusted once every 10 s of real time The simulation was run for 6 d of real time, to give the data of Table 1 All correlations, real and modelled, are associated with a probability of $P < 0\ 01$ Considering the simplified picture of the total energy flow and the considerable assumptions underlying the estimates of flow on which the calculation is based, the agreement with the food intake characteristics of real rats is reasonable It may be seen that there is a significant correlation between the size of a meal and the time to the onset of the next meal Correlations of meal size to premeal interval are smaller and less reliable statistically in both real and modelled rats

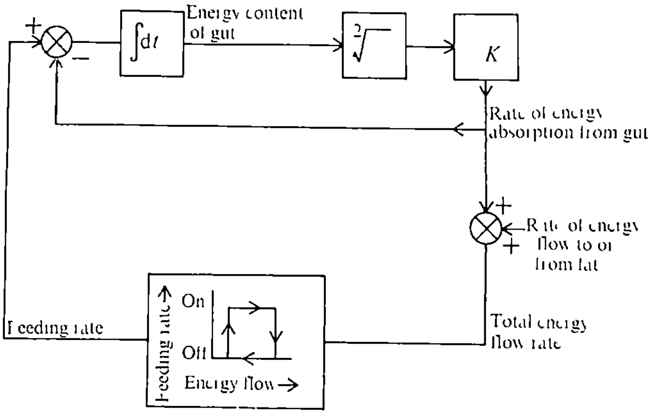

Fig 1 A model of feeding

The success of the simulation is not a confirmation of simple homeostatic concepts, for the model is based on rates of energy flow, not directly on amounts of energy eaten or stored Further preliminary work suggests that the model can be extended to include estimates of increments in body energy which would link short term control to long term regulation

Where the physiological data are available, conditions other than *ad libitum* maintenance of intact animals can be modelled Indeed, an homologous model can be constructed for man Hitherto, models of the control of food intake have not been based on physiological data[19], have been unprogrammable and non-predicting block diagrams[10,20–22] or have been computerised by postulating unmeasured variables and deciding parameters by fiat[23] The conditions simulated to date using the present model have generated realistic predictions The dynamic and static phases of the hyperphagia which follows lesions of the ventromedial hypothalamus have been successfully simulated[24] This implies that the hyperphagia is secondary to hormonal abnormalities The feeding pattern of the recovered lateral hypothalamic aphagic rat is mimicked by the model, if feeding rate is substantially slowed This suggests that disruption of oral reactions is primary in the residual deficits of this condition

The chronically diabetic rat has been modelled on the basis that in this condition there is no net storage or mobilisation of fat and only one-third of the ingested energy is able to act on receptors suppressing feeding[25,26] The model correctly predicts a food intake of about 60 g per 24 h The real rat, however, takes the food in many meals distributed throughout the day and night[25] whereas the model of Fig 1 predicts a single very prolonged meal in each lighting phase A simple addition, that of an inhibitory pathway from stomach stretch receptors to the hunger system[3] brings the model into agreement with reality

Table 1 Feeding pattern parameters in real and modelled rats (means ± s d)

	Real rat			Modelled
	A*	B†	C†	rat
Dark Phase				
Total intake (g in 12 h)	13 0	17 3±3 4	14 2	13 3±1 6
Size of meal (g)	2 6	3 1±2 2	2 9	2 1±0 4
Meal to meal interval (min)	137	160	—	100±15
Meal size/post-meal interval				
ratio	27 8	—	—	20±3
correlation	—	0 84	—	0 76
Light Phase				
Total intake (g in 12 h)	8 5	5 1±1 3	7 0	6 4±0 9
Size of meal (g)	2 0	1 6±1 2	2 3	2 2±0 9
Meal-to-meal interval (min)	154	218	—	282±73
Meal size/post-meal interval				
ratio	14 3	—	—	9±0 6
correlation	—	0 65	—	0 98

*Ref 14
†Ref 17

Even in the present single-factor theory of feeding control, many components interact The verbal theories currently extant, and even intuitions concerning the predictions of quantitative theories such as the present one, can only be evaluated decisively by specification as a computer model

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Comparative activity of red cell adenosine deaminase allelic forms

CERTAIN enzymes have allelic forms which can be distinguished electrophoretically The variability of the structural genes determining these enzymes[1] is one of the most challenging problems in modern biology, since no particular reason can be found for their selection during evolution and so they form the main subject of debate between the 'pan-neutralists' and the 'pan-selectionists' To try and throw some light on this problem we have compared the enzymic activity of the different electrophoretic forms of human red cell adenosine deaminase

Only a few enzymes have previously turned out to be suitable for this type of comparison[2,3]—those with at least two common electrophoretic phenotypes and a simple quantitative assay

Adenosine deaminase (ADA) presents an electrophoretically demonstrable polymorphism depending upon an autosomal gene with two common codominant alleles *ADA*^{1} and *ADA*^{2}, as shown by Spencer *et al*[4] A number of cases of ADA deficiency have also been reported[5] They were associated with a severe combined immunodeficiency disease[5], and a causal relationship between the enzyme deficiency and the immunological disease mediated by the resulting pyrimidine starvation has been suggested[6] (see also Giblett *et al*[7])

We devised a new, simple and reliable quantitative assay of the red cell ADA activity With this method we found that the mean activity of the red cell ADA in the ADA 1 is higher than that of the ADA 2–1 phenotype This shows that the activity associated with the *ADA*^{1} allele is greater than that associated with the *ADA*^{2} allele

A random sample of 196 undergraduate students (first year) of the University of Rome was studied The red cell ADA phenotypes were determined according to Spencer *et al*[4] The red cell ADA activities were all assayed within a few hours after bleeding The present method is an adaptation to red cell lysates of Kalchar's method[8] The lysates were incubated with adenosine in excess The reaction was stopped with perchloric acid and the quantity of adenosine consumed was estimated from the decrease of $E_{2,680}$ (inosine and hypoxanthine have the same $E_{2,680}{}^{\mathrm{Mol}}$ which is about half that of adenosine[9] In our hands the $\Delta E_{2,680}{}^{\mathrm{Mol}}$ of the transformation adenosine into inosine or hypoxanthine was $6\ 77\times10^3$) Linearities with time and haemolysate concentration were checked Haemoglobin concentration was determined with the

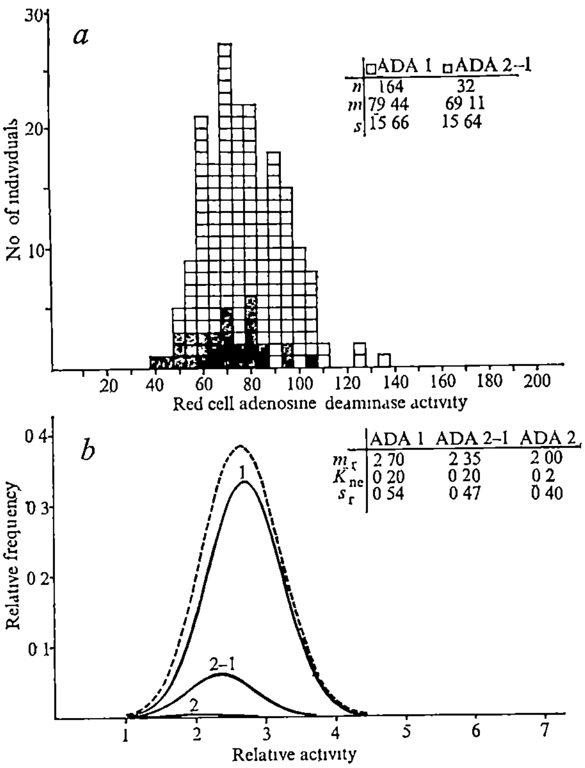

Fig. 1 Distribution of red cell adenosine deaminase activities *a*, Observed distribution in a sample of 196 random individuals *b*, Expected normal distributions in the ADA 2, ADA 2–1 and ADA 1 phenotypes with relative mean activities, m_r, 2 00, 2 35 and 2 70, respectively The coefficient K_{ne} of intraphenotypic variation, that is of the non-electrophoretic variation, is assumed to be 0 20 for all the three phenotypes The relative areas correspond to the expected relative genotype frequencies, $p^2=0\ 007$, $2pq=0\ 150$ and $q^2=0\ 843$

Packed red cells washed three times with isotonic saline were lysed by a triplicate cycle of freezing (in acetone and dry ice) and thawing The lysates were diluted 50 times with Sørensen phosphate buffer 1/15 M *p*H 7 5 preincubated at 37° C The reaction was started within 15 min (the enzyme activity is stable in these conditions for at least 1 h) by mixing 1 vol of lysate with 5 vol of a preincubated solution of 0 337 mM adenosine in the same buffer At 0 time and 75 min three aliquots were transferred into equal volumes of 8 7% PCA After at least 15 min the precipitates were spun down and 5 ml of the clear supernatants were neutralised with 0 35 ml of 5 M K_2CO_3, transferred into a freezer for about 10 min and filtered The $E_{2\ 680}$ were then determined Haemoglobin was determined in the assay mixture (adenosine+lysate)

The activity is expressed as μmol of adenosine consumed per h at 37° C per g of haemoglobin cyanmethaemoglobin method[10] The precipitation of proteins makes this method more laborious but more sensitive (a more concentrated lysate may be used) and less expensive (no need of xanthine oxidase) than that of Hopkinson *et al*[9]

Figure 1 shows the distributions of the red cell ADA activities observed in the two ADA phenotypes found in the present sample

The difference between $\overline{\text{ADA 1}}$ and $\overline{\text{ADA 2–1}}$ (the mean enzyme activities of the ADA 1 and ADA 2–1 phenotype, respectively) is statistically significant ($t=3\ 41$, $P<0\ 001$) in spite of the large intraphenotypic variation The enzyme activity (expressed as µmol of adenosine consumed per h at 37° C per g of haemoglobin) associated with the ADA^1 allele, estimated as $(\overline{\text{ADA 1}})/2$, is 39 77, that is, 35% higher than that associated with the ADA^2 allele (29 34) estimated by subtracting the ADA^1 activity to $\overline{\text{ADA 2–1}}$, on the assumption that no interaction occurs between ADA^1 and ADA^2 alleles in the heterozygote This seems to be a quite reasonable assumption, considering that no hybrid ADA molecules are present in the heterozygotes

A portion of the variation of the red cell ADA activity can thus be accounted for in terms of the difference existing between the activities associated with the two common electrophoretically distinguishable alleles of this enzyme This portion can be conveniently expressed as the corresponding partial variance, which is estimated to be, in our sample, 0 019 if one designates as 1 the activity associated with the ADA^2 allele (see Fig 1) This variance is in fact equal to $2pq\,d^2(1+K_{ne}{}^2)$ (see Modiano[3]) where $p(_{ADA}{}^2)=0\ 08$ and $q(_{ADA}{}^1)=0\ 92$ are the gene frequencies found in the present sample (which are similar to those previously reported for the same population[11]), d, the relative difference between the ADA^1 and ADA^2 activities, is 0 35 (see legend of Fig 2), and K_{ne}, the coefficient of the non-electrophoretic variation (standard deviation divided by the respective mean within each electrophoretic phenotype), is rounded to 0 20 for all the three ADA phenotypes Since the observed total variance was 0 282, it may be concluded that 0 019/0 282, that is about 7% of the total variation of the red cell ADA activity, is associated with the electrophoretically demonstrable genetic variation of this enzyme The nature of the remaining major portion of variation (the intraphenotypic or non-electrophoretic variation) remains unknown It is also unknown why the ADA 1 and ADA 2 allelic enzymes present different activities in the red cell lysates and whether or not this difference applies to other tissues as well These questions remain unanswered for most of the interallelic differences so far identified

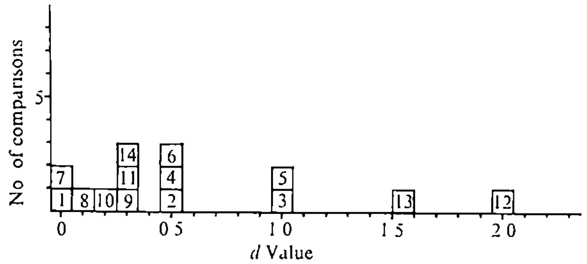

Fig 2 The distribution of the 14 relative differences, d, known at present between the enzyme activities associated with common electrophoretic alleles of red cell enzymes*

d is the relative difference between the enzyme activities associated with different alleles expressed in terms of the activity of the least active allele For example, if the absolute allelic P^a, P^b and P^c activities are 60, 90 and 120 as in the red cell acid phosphatase, their relative activities are 1 0, 1 5 and 2 0, respectively, with three d values $d_{P^b-P^a}=1\ 5-1\ 0=0\ 5$, $d_{P^c-P^a}=2\ 0-1\ 0=1\ 0$, and $d_{P^c-P^b}=2\ 0-1\ 5=0\ 5$ The comparisons refer only to those allelic enzymes whose associated activities have been compared because they were known to be electrophoretically different

A list of the 14 comparisons Each comparison is represented in the figure by the number in the corresponding box acid phosphatase[12 13] (1) P^r–P^a, (2) P^b–P^a, (3) P^c–P^a, (4) P^b–P^r, (5) P^c–P^r, (6) P^c–P^b, phosphoglucomutase[14,15] (7) $PGM_1{}^1$–$PGM_1{}^2$, glucose-6-phosphate dehydrogenase[16 17] (8) Gd^B–Gd^A, Adenylate kinase[18,19] (9) AK^1–AK^2, 6-phosphogluconate dehydrogenase[20–22] (10) Pgd^a–Pgd^b, Glutathione reductase[23] (11) $GSSG$–R^S–$GSSG$–R^F, Glutamic pyruvic transaminase[24,25] (12) Gpt^1–Gpt^2†, Uridine monophosphate kinase[7] (13) $UMPK^1$–$UMPK^2$, Adenosine deaminase (14) ADA^1–ADA^2 (this paper)

* See Modiano[3] for detailed references

† The difference in the figure is that reported by Chen *et al*[24]

Table 1 Non-random assortment* of the relative activities and frequencies of the common alleles of red cell enzyme polymorphisms

	Polymorphisms in which the most active allele is	
	the most common allele	the least common allele
Observed	12†	1‡
Expected	6 5	6 5

* The likelihood of a discrepancy from randomness like the one observed or greater, in either direction, is $2\,(13+1)\times(1/2)^{13}\simeq 0\ 003$

† The following is the list of these twelve red cell enzyme polymorphisms acid phosphatase[12 13] (the findings in Caucasians where the most common allele is the one with intermediate activity have not been considered informative), glucose-6-phosphate dehydrogenase[16 17], adenylate kinase[18,19], 6-phosphogluconate dehydrogenase[20–22], glutamic-pyruvic transaminase[24 25], galactose-1-phosphate uridyltransferase[26], nicotinamide adenine dinucleotide nucleosidase[27], phosphoglucomutase (PGM_2)[28], peptidase C[29], peptidase A[30 31], uridine monophosphate kinase[7], adenosine deaminase (this paper)

‡ Gluathione reductase[23]

The difference in activity between the different allelic forms is a common finding Figure 2 shows that, as a rule, common electrophoretic alleles are associated with considerably different ($d\simeq 0\ 6$) enzyme activities in red cell lysates (12 out of 14 comparisons)

Further evidence suggesting that the common structural gene variations are not neutral is the non-random relationship between the relative frequency and activity found among the common alleles of the red cell enzyme polymorphisms studied so far (see Table 1)

In vitro V_{max}, however, is a poor measure of the enzyme's function *in vivo* Significant progress along these lines may therefore be expected from interallelic comparisons involving more significant enzyme characteristics, such as K_m (in relation with the *in vivo* substrate concentrations) and the *in vivo* stability But so far, only glucose-6-phosphate dehydrogenase has been studied at such a level[33–36] The use of intact red cells under physiological or suitable stress (such as drug induced) conditions[37] or of other cells and tissues should also provide further rewarding information

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Population size and natural selection in the land snail *Cepaea nemoralis*

ALTHOUGH most investigators have concluded that protein polymorphisms are maintained by balancing selection[1,2], some contend that they are the consequences of random drift of neutral or nearly neutral alleles[3,4] Many empirical data seem to support the selectionist view, although the mechanism that might be involved is not well understood In any case, critical data on changes in allelic or genotypic frequencies through time are not available for most natural populations Therefore, the major evidence for selective responses is provided by deviations from expected zygotic proportions at some given time

Studies of laboratory populations have indicated that some enzyme polymorphisms are examples of heterosis (overdominance), since experimental results consistently show heterozygote excesses over expected values[5,6] Data from natural populations are, however, harder to interpret[7,8] For example, heterozygote deficiencies can result from inbreeding or disruptive selection, or they can be an artefact caused by small sample sizes, scoring errors, sampling before completion of selection[9] or inappropriate pooling of subpopulations Although data on the size and spatial structure of the populations studied would often facilitate interpretations of genetic results, they are usually not available and with certain organisms are almost impossible to obtain[7] I have now obtained such data for populations of the helicid land snail *Cepaea nemoralis* and found that heterosis maintains polymorphisms at two enzyme loci

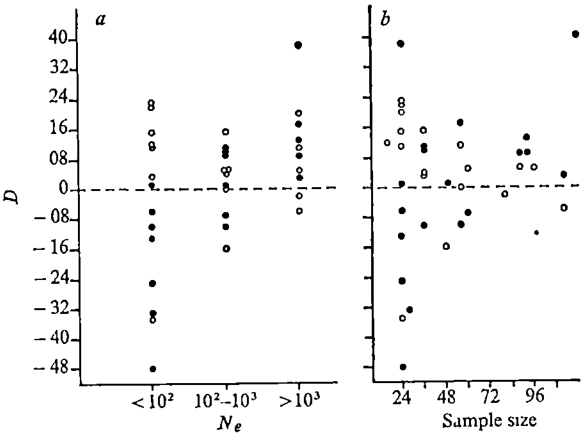

Fig 1 *a*, Effect of adult population size (N_e) on heterozygote deviations (D) at the *PGM-2* and *LAP-2* loci in *C nemoralis* The zero line represents exact Hardy-Weinberg equilibrium where $D = H_o - H_e/H_e = 0$ Heterozygote deficiencies appear below the zero line, excesses above it *b*, Effect of sample size on heterozygote deviations at the *LAP-2* (○) and *PGM-2* (●) loci. See text for further explanation

Because of their highly polymorphic shell patterns, populations of *C nemoralis* have been subject to evolutionary study in Europe (see ref 10 for a review) for decades The species has been introduced into North America on several occasions, and colonies are now found in various localities in the eastern United States and Canada They rarely occur outside towns or other habitats perpetuated by human disturbances The colonies are either subdivided into small, local populations, or may occupy areas much larger than the estimated panmictic unit of the snails[11] For this reason, the snails provide excellent opportunities for studying the effects of spatial patterning and population size on genetic variation

In addition to their shell colour and banding polymorphisms, *C nemoralis* populations found in the Piedmont and Great Valley of Virginia are polymorphic at one phosphoglucomutase locus (*PGM-2*), and at one leucineaminopeptidase locus (*LAP-2*) *PGM-2* has two alleles and *LAP-2* usually has three The enzymes provide clear electrophoretic patterns with good separation of all allelic variants Thus, the possibility of scoring errors is remote The genetic bases for the observed variation have been verified experimentally[12]

To study the effects of spatial patterns and effective population size (N_e) on genetic variability in *C nemoralis*, 303 immature individuals 9 mm or more wide and 586 mature specimens were collected from 18 Virginia localities between June 1972 and October 1973 The area occupied by the snails was measured or estimated at each site

Although *C nemoralis* is monoecious, it is an obligate outcrosser Therefore, the maximum N_e of a population approximates the numbers of mature individuals in that population[11] It is difficult to estimate the number of snails in natural populations since they move around too slowly for ordinary capture–recapture methods, and their patchy distribution makes quadrat or transect counts unreliable[15] Small areas, however, could be searched completely, and estimates of N_e in these populations were obtained by direct count Few mature specimens could have been

Table 1 Allele frequencies, heterozygote deviation, and sample size at the *LAP-2* and *PGM-2* loci in *C nemoralis* from 18 local populations in the Piedmont and Great Valley of Virginia

		LAP-2					*PGM-2*				
		f(a)	f(b)	f(c)	D	Sample N	f(a)	f(b)	D	Sample N	N_e
Lynchburg	1	0 47	0 07	0 46	−0 06	113	0 65	0 35	0 03	112	> 1,000
	2	0 10	0 07	0 83	0 03	36	0 49	0 51	−0 10	35	< 100
	3	0 24	0 01	0 75	0 15	36	0 44	0 56	0 11	36	100–1,000
Staunton	1a	0 01		0 99	0	57	0 90	0 10	−0 10	57	100–1,000
	2	0 31	0 06	0 63	0 20	24	0 71	0 29	0 38	24	> 1,000
	3	0 21	0 04	0 75	0 05	60	0 79	0 20	−0 07	60	100–1,000
	4	0 53	0 21	0 26	0 04	36	0 47	0 53	0 10	36	100–1,000
Harrisonburg	2	0 11		0 89	0 11	56	0 49	0 51	0 17	56	> 1,000
Lexington	1	0 27	0 01	0 72	−0 02	79	0 48	0 52	0 13	91	> 1,000
	2	0 25	0 03	0 72	0 05	88	0 41	0 59	0 09	89	100–1,000
	3	0 23	0 01	0 76	−0 16	47	0 48	0 52	0 01	49	100–1,000
	4	0 33	0 04	0 63	0 23	24	0 60	0 40	−0 06	24	< 100
	5	0 29	0 04	0 67	0 22	24	0 43	0 57	−0 13	23	< 100
	6	0 25		0 75	−0 35	24	0 27	0 73	−0 48	24	< 100
	7	0 17	0 25	0 58	0 15	24	0 44	0 56	−0 25	24	< 100
	8	0 25	0 15	0 60	0 11	24	0 58	0 42	0 01	24	< 100
	9	0 20	0 10	0 70	0 12	15	0 43	0 57	−0 33	27	< 100
	10	0 33		0 67	0 05	95	0 22	0 78	0 09	93	> 1,000
Total						862				884	

N_e is given for each population

overlooked, and this procedure for determining N_e in small populations is probably less biased than any other

Numbers of adult snails in the larger populations were estimated by a time-unit removal method The principle is that if a known number of snails is removed from a designated area in a fixed time, the number found subsequently is affected The rate at which catches fall off (a geometric series) is directly related to the size of the total population and the number previously removed[14] By taking samples from relatively large plots (9 m^2), I minimised the problem of temporary patchiness about single plants or other objects, and average density estimates of mature individuals were reasonably consistent from plot to plot within a population Average numbers of adult snails per unit area were then multiplied by the total area occupied by the population to estimate maximum N_e The size of the panmictic unit in large populations of *C nemoralis* has been estimated to be more than 1,000 individuals[15] My own unpublished data for North American populations confirm this

I used the horizontal starch-gel electrophoresis techniques of Selander *et al*[16], modified as described before[12] Allele frequencies, determined by direct count from the phenotypes observed on the gels, were used to calculate the expected genotypic frequencies by Levene's[17] method for small samples Deviations of observed from expected values were tested for significance using the G test for goodness of fit[18] For statistical analysis $LAP\text{-}2^b$ was pooled with the second least common allele in most samples There were departures from Hardy-Weinberg expectation in all cases except one (*LAP-2*, Staunton 1a, see below), though statistical significance was not noted in any

Heterozygote deviation (D) was calculated by the formula

$$D=(H_o-H_e)/H_e$$

where H_o and H_e are the observed and expected numbers of heterozygotes, respectively Table 1 lists the sample sizes, allele frequencies and values of D for both loci, and the estimated size of N_e

At the *LAP-2* locus, 13 of 17 populations showed a heterozygote excess, which was unlikely by chance alone ($P=0$ 025, sign test) (The eighteenth population, Staunton 1a, was not considered since the only variant in it was one $LAP\text{-}2^{a/c}$ heterozygote) This result does not conform to the hypothesis of neutral alleles, which would predict a statistically equal number of heterozygote excesses and deficiencies The simplest explanation for the heterozygote excess is some sort of balancing selection which results in some degree of superior heterozygote survival

On the other hand, at the *PGM-2* locus seven of the 18 populations showed a heterozygote deficiency, indicating no overall tendency toward heterozygote excess ($P=0$ 24, sign test) However, if the populations are grouped according to size, six of seven populations with N_e estimated to be less than 100 show heterozygote deficiencies, while nine of 11 populations with N_e greater than 100 show heterozygote excess These differences from expectation are significant ($P<0$ 025, Fisher exact test), and indicate a strong interaction between heterozygosity and population size (Fig 1*a*)

No interaction is evident between sample size and the direction of D, although, as would be expected, values of D tend to have higher variances at lower sample sizes (Fig 1*b*)

The findings suggest that the *LAP-2* and *PGM-2* polymorphisms are maintained in these natural populations by heterosis Selection for heterozygotes may be stronger at the *LAP-2* locus, since heterozygote excesses are present even in populations with small N_es A possible explanation is that LAP catalyses the degradation of a diversity of substrates, some of which presumably enter the snail from the environment[19] Individuals heterozygous at this locus may be able to utilise a greater diversity of energy or nutrient sources by virtue of having two forms of the enzyme An alternate explanation is that this locus is closely linked to another which is subject to heavy selection

PGM, on the other hand, has only been shown to catalyse the conversion of α-D-glucose-1-phosphate to α-D-glucose-6-phosphate The apparently milder heterotic effects may result from favourable interactions with other gene products rather than from a capability to handle a greater variety of substrates The validity of such speculations can only be established when the functional properties of the electrophoretic variants are understood In any case, these data indicate that the effects of stochastic processes in small populations are strong enough to override any heterozygote advantage, either at this locus or at any closely linked to it

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Alternate host for mosquito parasite *Coelomomyces*

THE water mould *Coelomomyces* (Chytridiomycetes, Blastocladiales) is an obligate parasite of mosquito larvae The fungus develops in the haemocoel of the host and is first visible as small unwalled hyphal bodies, which later differentiate into masses of thick-walled resistant sporangia In appropriate conditions, each sporangium releases a large number of posteriorly uniflagellate zoospores[1] Field studies of this pathogen indicate that it may be responsible for significant epizootics of mosquito larvae and considerable interest has been generated over its possible use in biological control of these troublesome insects[2-6] This potential has not been confirmed in the laboratory The production of infected larvae in controlled environmental conditions has proved to be a difficult and erratic task[1,7] Couch[8] has devised a technique for the production of large numbers of infected mosquitoes but this method uses quantities of microalgae and water from the original infection site

Our studies have focused on *C psorophorae* in *Culiseta inornata* The fungus was first discovered in 1956 in the irrigated districts of southern Alberta[5] It has reappeared every year since that date and has often induced high mortalities in the mosquito populations Attempts to establish infected colonies in the laboratory encountered the same problems noted by earlier workers Limited infection was obtained by the Couch method, but only after adding water, algae and other substrates from the Canadian collection site Efforts to increase the infection rate by modifying first the environment (for example, *p*H, temperature, water character, light and redox potential), second the number or condition of the host and, third, the quantity of fungal inoculum, were inconclusive This work did permit the removal of algae from the infection trials but did not materially improve their reliability

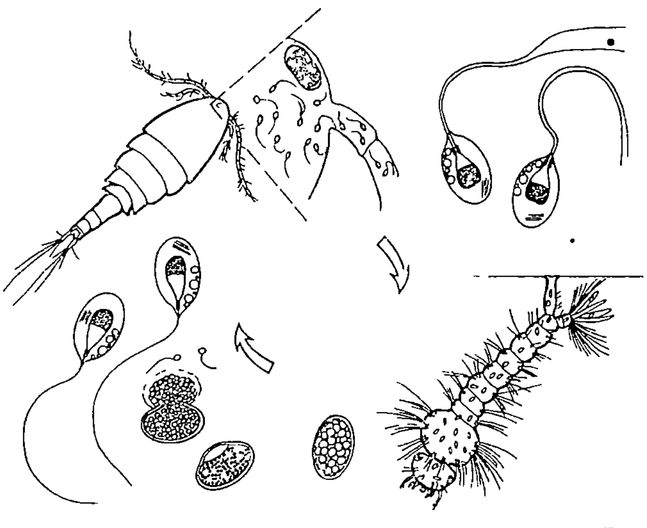

Fig 1 Life history of *Coelomomyces psorophorae* Resistant sporangia from the body of the mosquito release zoospores which infect *Cyclops* Biflagellate spores from the *Cyclops*/*Callimastix* phase infect mosquito larvae

Attempts to infect mosquitoes in simple, clean systems using synthetic pond water, and clean fungal inoculum failed repeatedly Sequential addition of individual components from our 'gross' infection pans to our 'clean' trials has provided the key to the infection problem Addition of the copepod *Cyclops vernalis* to pans containing fungi and mosquito larvae resulted in the appearance of infected mosquitoes Control pans lacking copepods have never yielded diseased larvae, in spite of the addition of masses of zoospores and/or sporangia This implied that *Coelomomyces* had an alternate, copepod host To test this hypothesis, washed copepods, from pans with a record of high infectivity, were combined with mosquito larvae in synthetic pond water Within a few days, 40% of these larvae were obviously infected with *C psorophorae*

Examination of the copepods revealed the presence of an 'unknown' phase in the life history of *Coelomomyces* The body cavities of the diseased *Cyclops* were packed with swarming zoospores At escape, the spores were found to be predominantly biflagellate, but otherwise similar to the zoospores produced in the resistant sporangia of *Coelomomyces* Mosquitoes exposed to a suspension of these spores displayed typical *Coelomomyces* hyphal bodies and sporangia within a few days of inoculation

These results indicate that the life history of *C psorophorae* includes two different hosts (Fig 1) Attempts to infect mosquitoes with spores from the resistant sporangia produced in the mosquito or to infect copepods with spores from other infected *Cyclops* have consistently failed and it seems that alternation of different hosts is obligatory Such heteroecism is common in rust fungi, but has not been previously reported for the Chytridiomycetes or other phycomycetes

The copepod phase of the fungus resembles the *Cyclops* parasite *Callimastix cyclopis* Weissenberg[9] Originally described as a protozoan parasite, it has more recently been allied to the Blastocladiales[10,11] The zoospores are characteristically multiflagellate, with all the zoospores from one copepod bearing the same number of flagella (for example, 2–5 per spore) The zoospore of *Callimastix* carries a peculiar paracrystalline body[11], which is also found in *Coelomomyces*[12,13], but has not been reported from other fungal zoospores The ultrastructure of the unwalled plasmodia and spores, the swarming of the spores in the host body, and the general morphological development of the copepod phase of *Coelomomyces* indicates that this phase may be considered as a biflagellate species of *Callimastix* As the latter genus was described earlier than *Coelomomyces*[14] it would seem to have nomenclatural priority

The discovery of heteroecism in *Coelomomyces* has obvious implications for its possible use in biological control of mosquitoes Whether other species and strains of *Coelomomyces* and *Callimastix* have complementary hosts requires further study It is interesting to note, however, that in the earlier *Callimastix* studies, infected *Cyclops* were obtained from field collections or gross cultures and Vavra and Joyon[11] speculate on an unknown phase in the development of their copepod parasite

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Abscisic acid levels in relation to drought tolerance in varieties of *Zea mays* L.

THE level of growth inhibitor in plants increases when they are subjected to water stress and the major constituent of this inhibitor is abscisic acid (ABA)[1-3] By its effect in closing stomata, ABA can control the rate of transpiration[4-7] and it is therefore likely that ABA may be involved, to some extent, in the mechanism conferring tolerance of plants to drought

Palacios[8] and Muñoz[9] have developed a line of maize (Latente) which shows enhanced ability to withstand drought conditions Seed of this line was obtained from Mexico and it was decided to investigate whether its higher resistance to water stress was related to a capacity to produce appreciable quantities of abscisic acid Accordingly we have compared the levels of ABA in detached leaves of Latente with those of two European varieties (Anjon 210 and LG 11) which have not been bred for tolerance to drought

Seeds of each variety of *Zea mays* L were sown in vermiculite and the seedlings raised in a growth room with a 15 h photoperiod (Mazda 'daylight' fluorescent tubes) at 21 5° C and a minimum relative humidity of 75% At the end of the dark period on day 12, by which time ligules from the second leaves were exposed, 25 g batches of leaves were collected by excision 2 mm above the coleoptiles and laid on sheets of dry filter paper in a glass container Warm air (35 ± 2° C at leaf surface) from three hair driers was directed across the leaves until a loss of 10% in fresh weight had occurred (about 10 min) The leaves were then placed for 4 h in a closed glass chamber on four thicknesses of filter paper moistened with 20 ml distilled water The controls were not subjected to the water stress treatment but were kept at the same high relative humidity for 4 h At the end of this period, treated and control leaves were cut into small pieces and extracted in methanol for 48 h at—20° C After the removal of the solvent under reduced pressure, the extract was partitioned twice between 2% sodium bicarbonate and redistilled ether The aqueous fraction was washed with more ether and then acidified to *p*H 2 8 with 2 N H_2SO_4 The free acidic compounds were extracted with ether and the extract dried over anhydrous sodium sulphate Most of the solvent was then removed

Table 1 Determination of free ABA in detached leaves of wilted and non-wilted maize seedlings

	Free ABA (µg equivalents)					
	Latente		Anjon 210		LG 11	
	Control	Wilted	Control	Wilted	Control	Wilted
Experiment 1	12 8	50 3	2 4	21 1	3 1	11 8
Experiment 2	13 5	50 2	2 2	16 4	2 5	26 0
Mean	13 1	50 2	2 3	18 7	2 8	18 9

Data are from two independent experiments in which a Mexican line (Latente) and two European varieties of maize (Anjon 210 and LG 11) were grown in vermiculite

Further separation was carried out on 20×20 cm fluorescent silica-gel coated TLC plates (Merck GF_{254}, 0 25 mm thick), which were developed in *n*-propanol–*n*-butanol–0 88 ammonium hydroxide–water (6 2 1 2 v/v) (ref 10) The zone on each plate corresponding to an ABA marker spot (ultraviolet identification) was scraped from the chromatogram and eluted with methanol Each eluate was concentrated for the second TLC stage and developed six successive times in chloroform–benzene–acetic acid (100 100 1 5 v/v) The silica in the ABA region was then removed and eluted with methanol Final purification was carried out by ascending paper chromatography (Whatman No 1) using *iso*propanol–0 88 ammonium hydroxide, water (8 1 1 v/v) and that part of the paper corresponding to the ABA zone (R_F 4 5–8 5) was divided into eight equal segments These were assessed for inhibitory activity in the wheat coleoptile straight growth test[11] The mean length of the ten coleoptile sections used in each assay was expressed as a percentage of the length of the controls A dose-response curve was obtained for synthetic (±) ABA so that the inhibitor extracted from maize could be expressed as µg equivalents of ABA (ref 3)

The free ABA in detached non-wilted maize leaves in Latente, which is tolerant to water stress, was found to be more than four times that found in the less tolerant varieties Anjon 210 and LG 11 On subjecting the detached leaves to water stress the level of ABA increased in all varieties, confirming similar observations by Wright[1] and Wright and Hiron[3] (Table 1)

Although water stress produced a greater relative increase of ABA in the varieties Anjon 210 and LG 11 than in Latente, the level of ABA in the wilted Latente leaves was much higher Such high levels would be expected to have a profound effect in closing leaf stomata thereby reducing transpiration and protecting the plant against water loss Not only this, but the well-established effects of ABA in inhibiting the activity of growth hormones would be expected to operate strongly, thereby restricting growth and conserving energy normally utilised in growth processes Our results indicate that such protective mechanisms may be more well developed in the drought tolerant Latente maize than in the other two varieties studied

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Altered fucolipid patterns in cultured human cancer cells

STUDIES in this laboratory[1] have shown that fucolipid metabolism is markedly altered in membranes of cells transformed by oncornaviruses Transformed cells exhibit a sharp decrease in radioactive fucose incorporation into chromatographically less mobile, presumably more complex, fucolipids with a corresponding rise in radioactivity in more mobile fucolipids Studies of fucolipid metabolism in normal rat kidney (NRK) cells transformed by a cold-sensitive transformation mutant of murine sarcoma virus (NRK (MSV-1b)) revealed a correlation between the appearance of the transformed phenotype and altered fucolipid synthesis[2] The fucolipids of SV40 and herpes simplex virus-transformed cell lines have also been examined[3], some of the transformed lines were oncogenic while others were not when injected into appropriate animals The cell lines which were capable of producing tumours had a block in the synthesis of the complex fucolipids comparable with that observed in the oncornavirus-transformed cells In contrast, the transformed cell lines that did not produce tumours showed only a minor block in the formation of the complex fucolipids

We have now examined the fucolipids of a number of cultured human tumour cell lines[5] The results indicate a block in synthesis of the complex fucolipids that is comparable with that observed in virus-transformed cells

The human tumour cell lines used included those isolated and characterised by Giard *et al*[4] A-375, malignant melanoma A-204, rhabdomyosarcoma, and A-549 lung carcinoma In studies[4] with mice treated with antithymocyte serum (ATS), A-375 produced rapidly growing subcutaneous tumours resembling amelanotic melanomas, A-204, slowly growing sarcomas, and A-549, rapidly growing subcutaneous and intracerebral carcinomas Additional cell lines included SV40-transformed human cell line (SV80)[5] and 8387, a human fibrosarcoma cell line[6] In addition, several strains of normal human embryonic cells were examined The cells were grown in glass bottles in Eagle's minimal essential medium (MEM) supplemented with 10% foetal calf serum, and were labelled 3–5 d after plating with 1-^{14}C-fucose (0 5 μCi ml^{-1}, 57 mCi mmol^{-1}) or with 1-^{3}H-fucose (5 μCi ml^{-1}, 1 4 Ci mmol^{-1}) Cell monolayers were washed three times with cold phosphate-buffered saline (PBS), removed by scraping into PBS and sedimented to a pellet The pellet was washed with PBS and the lipid was extracted[1]

Chromatographic analysis of the fucosylglycolipids was accomplished by thin-layer chromatography (Q-5 plates, Quantum Industries, Fairfield, New Jersey) in 2-propanol NH_4OH H_2O (7 2 1, by volume) The amount of radioactivity was estimated by scraping the plate in 1-cm strips from origin to solvent front and counting the fractions in a scintillation spectrometer[1] When ^{14}C-fucose-labelled lipid was examined, the plates were visualised, before scraping, by exposure to Kodak No-screen X-ray film

It seemed unlikely that nonlipid contaminants (that is fucose, GDP-fucose, fucose-1-phosphate or fucopeptide) might have been present in the lipid extract The materials characterised as fucolipids did not dialyse, were unaffected by snake venom phosphadiesterase or by *Escherichia coli* alkaline phosphatase, and did not have the same R_F values as those of authentic fucose, GDP-fucose or fucose-1-phosphate These properties argue against the possibility of contamination with the small molecular weight material indicated above Further, chromatography of the cytosol fucose radioactivity did not reveal components with the same R_F values as those of the fucolipids, whereas the lipid extract from a thoroughly washed high speed pellet yielded the identical fucolipid patterns as those obtained from

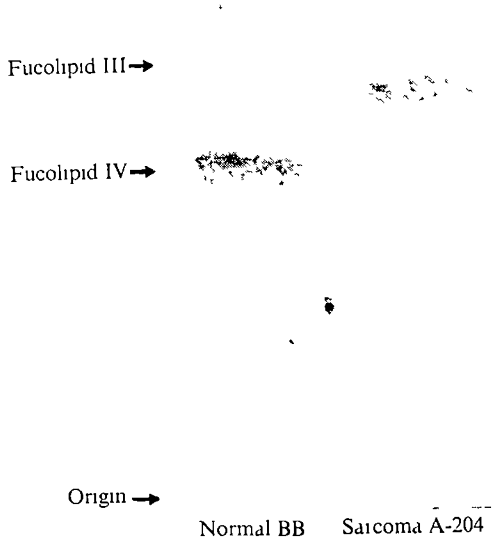

Fig 1 Autoradiogram of one dimensional chromatograms of ^{14}C-fucose-labelled lipids of BB (normal human embryonic lung) and A-204 (sarcoma) cells Cells were cultured in medium supplemented with ^{14}C-fucose as described in the text, as are the conditions for collecting, extraction and chromatography of the fucolipids The figure is a composite of autoradiograms of two thin-layer plates, 5×20 cm, chromatographed in parallel, solvent was $CHCl_3$ CH_3OH NH_4OH (7 2 1, by volume) Fucolipids III and IV are shown, the more chromatographically mobile fucolipids (I and II) are not visible because of the relatively low level of radioactivity (see Table 1) in these components

Table 1 Incorporation of ^{3}H-labelled fucose into lipids of normal human embryonic lung (BB) and human tumour cell lines

Fucolipid	BB (normal)*		8387		A-204		SV80		A-549		A-375	
	%	c p m per mg protein	%	c p m per mg protein	%	c p m per mg protein	%	c p m per mg protein	%	c p m per mg protein	%	c p m per mg protein
I	2 8	166	6 8	494	6 7	394	1 8	167	10 3	506	16 3	852
II	8 3	491	11 0	799	10 7	630	3 6	333	14 7	722	24 5	1,281
III	16 7	987	21 9	1,591	80 0	4,711	73 2	6,772	50 0	2,457	44 9	2,347
IV	72 2	4,269	60 3	4,381	2 7	159	21 4	1,980	25 0	1,228	14 3	748

Each cell line was grown for 72 to 96 h (to obtain low and high cell density) in the presence of ^{3}H-fucose (5 μCi ml^{-1}, 1 4 Ci mmol^{-1}) Conditions for collecting and for lipid extraction are indicated in the text

* The fucolipid pattern of many different strains of normal human embryonic cell lines were studied, with comparable results to those seen for BB cells

extracted whole cells In addition, the fucolipids did not bind to DEAE-cellulose, which not only rules out negatively charged small molecular weight material (that is fucose-1-phosphate, GDP-fucose) but also makes it less likely that the compounds were fucoprotein Further evidence to rule out the possibility of fucoprotein (or fucopeptide) included total resistance to exhaustive pronase digestion It is also noteworthy that the Sephadex G-50 elution profiles of the major fucolipids (that is III and IV below) failed to manifest any overlapping with fucopeptides prepared according to Buck *et al*[10] We feel that the above evidence strongly supports the lipid nature of the fucose-labelled components Nevertheless, we are attempting to accumulate a sufficient amount of these lipids, which are present in extremely low amounts, to carry out definitive chemical analyses

Figure 1 is an autoradiogram of a one-dimensional chromatogram of the ^{14}C-fucose-labelled lipids of normal human embryonic cells (Fig 1, lane 1) and of cultured human rhabdomyosarcoma (A-204) cells (Fig 1, lane 2) Approximately equivalent amounts of radioactivity were chromatographed with each sample It is apparent that the least chromatographically mobile fucolipid (that is FucCer IV) is greatly reduced in the rhabdomyosarcoma culture In a comparable experiment the alternative isotopic form of fucose (^{3}H) was used to label the fucolipids of a number of human tumour cell lines, including those in Fig 1 The results (Table 1) show that four of the tumour cell lines had a significant reduction in the incorporation of ^{3}H-fucose into fucolipid IV, the chromatographically least mobile component In contrast, its incorporation into fucolipid IV in the 8387 cell line obtained from a fibrosarcoma was similar to that for the normal cell line The experiment presented in Table 1 is representative of three separate experiments, each done in duplicate In addition, comparable results to those seen in Table 1 were observed when ^{14}C-fucose was used with the same cell lines (Fig 1) Several other normal human embryonic cell strains gave patterns similar to those shown for BB cells

It has been shown that the membranes of human adenocarcinomas lack the blood group haptens A and B but accumulate the Le^{a} and H haptens[7] More recently, Stellner and Hakomori[8] demonstrated that the altered fucosylglycolipid patterns (that is blood group determinants) in adenocarcinoma tissue are due to a deficiency in glycosyltransferases rather than to enhanced hydrolase activity Likewise, Fishman *et al*[9] noted the absence of a specific ganglioside galactosyltransferase in mouse cells transformed by murine sarcoma virus

One of the problems in examining human tumour material is the difficulty of obtaining adequate normal control cells Although we have not resolved this issue, it is noteworthy that regardless of the tissue source (kidney, lung or embryo) of the normal cells studied, or the cell type (epithelial or fibroblastic), or the species of primate from which the cells were derived, the fucolipid pattern was constant.

In the present study, altered patterns of incorporation of isotopically labelled fucose into the fucolipids of several types of cultured human tumour cells were observed The altered fucolipid patterns probably reflect changes in the glycosyltransferase activities similar to those reported[8] for adenocarcinoma tissue It is noteworthy that the fucolipid changes observed in the human tumour cells were similar to those observed in oncornavirus-transformed cells and in DNA-transformed, oncogenic cells Three of the human tumour cell lines (A-204, A-549 and A-375) had been found to produce tumours in ATS-treated mice[4] The cells of the 4th line, 8387, were fibroblastic in nature, although they piled up more than fibroblasts derived from healthy tissue, however, they failed to grow in ATS-treated mice[6] In this manner, the 8387 line acted like those SV40- and herpesvirus-induced cell lines that are transformed but not oncogenic[3] The SV80 line produced tumours in ATS-treated mice but they were then rejected, perhaps because SV40-transformed cells contain stronger antigens than spontaneous tumours[5]

These studies on human cancer cells in culture are built on our previous investigations of virus-transformed lines They offer a much needed biochemical method for identifying human cell lines that remain cancerous after being grown in culture

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Essential fatty acids in cystic fibrosis

THE nature of the metabolic defect in cystic fibrosis (CF) has, to date, not been elucidated. Elliott[1] reported that intravenous administration of soybean oil and lecithin to children suffering from CF caused their sweat sodium concentration to decrease towards normal values. This prompted us to investigate the fatty acid spectra of the various serum lipid classes of children with CF. We have found that affected children are deficient in certain essential fatty acids.

Table 1 Fatty acid spectra of serum lipid classes of children with cystic fibrosis and of normal children

Fatty acid	Cholesteryl ester		Triglyceride		Phospholipid	
	CF*	N†	CF	N	CF	N
12:0	Trace	Trace	Trace	Trace	—	—
14:0	1.3±0.07	0.9±0.02‖	1.9±0.22	1.8±0.20	Trace	Trace
16:0	25.3±0.95	22.6±1.30	40.9±1.00	38.1±0.33‡	43.1±0.80	43.6±1.71
16:1	7.0±0.89	2.7±0.43¶	5.0±0.39	2.0±0.13‖	Trace	Trace
18:0	7.5±0.94	4.6±0.73‡	7.4±0.48	15.4±2.00¶	25.1±0.58	21.7±1.44
18:1	32.2±1.34	27.1±0.81§	41.4±1.01	37.2±0.94§	20.5±1.13	14.0±0.95‖
18:2	23.3±3.22	38.6±2.88¶	3.5±0.32	5.3±0.97	6.4±0.32	13.0±0.69‖
20:4	3.2±0.33	3.4±0.90	—	—	4.9±0.36	7.7±0.68¶

CF, cystic fibrosis, N, normal. Figures give percentages of fatty acid present ± s.e.

* Five cases (four female, one male)

† Four cases (three female, one male)

‡$P = <0.05$

§$P = <0.02$

¶$P = <0.01$

‖$P = <0.001$

Sera were obtained from five children (aged 4.5–12.5 yr) with CF, and compared with sera from four normal children (aged 5–12 yr). Serum lipids were extracted with chloroform–methanol (2:1)[2] and subjected to thin-layer chromatography on silica gel G using hexane–ether–glacial acetic acid (first 60:40:1 and then 90:10:1) as developing solvents[3]. The lipid classes were visualised on the plates with iodine vapour[4] and after sublimation of the iodine, the appropriate areas were scraped, and the lipids were eluted and transesterified with a BF_3–methanol mixture. The resulting fatty acid methyl esters were analysed by gas–liquid chromatography using a column of 16% diethylene glycol succinate on 100–120 mesh Gas-chrom P. An argon ionisation detector was used. The column was standardised for retention times and quantitated at 175° C and 20 lb inch^{-2} of argon gas using purified standards.

The results are summarised in Table 1. There are significant differences between CF and normal sera in all the classes of serum lipids analysed. In the cholesterol esters, the CF sera contained significantly more myristic, palmitoleic stearic and oleic acids and significantly less linoleic acid. In the triglycerides the CF sera were richer in palmitic, palmitoleic and oleic acids but contained less stearic acid. Serum triglycerides of CF children contained 34% less linoleic acid but the difference was not statistically significant. In the phospholipids, the CF sera contained significantly less linoleic and arachidonic acids and more oleic acid. Kuo and Huang[5] found significantly less stearic and linoleic acids in the serum free fatty acids of CF children and significantly more palmitoleic acid and acids of chain length below that of palmitic acid (presumably myristic acid). Caren and Corbo[6] found that the major change in the free fatty acid fraction of CF children was in the large increase in palmitoleic acid. Our data confirm their other findings which were assessed from the standpoint of relating CF to impairment of liver function.

The data suggest another approach to the study of the metabolic defect in cystic fibrosis. There is an apparent deficiency in essential fatty acids, and a marked reduction in serum vitamin E levels has also been reported in CF[7]. These conditions might be partially corrected by intravenous injection, or possibly feeding of, lipids containing essential fatty acids with concomitant administration of vitamin E. The observed deficiency in essential fatty acids may be produced by a reduced ability to absorb these acids from the diet and this, in turn, may result in defects in membrane structure or stability. Reduced levels of vitamin E in CF may reflect a reduced requirement for this vitamin because of the lower levels of essential fatty acids. It is also possible that the observed deficiency in serum levels of essential fatty acids may lead to less than normal production of prostaglandins. These approaches are under investigation.

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Alteration of fibroblast gene products *in vitro* from a subject with Werner's syndrome

CULTURED human fibroblasts have a limited replicative capacity[1] and provide an excellent model for the study of biological ageing[1-4]. Recent studies have demonstrated that toward the end of their replicative lifespan, normal fibroblasts accumulate a significantly higher proportion of defective enzymes and simultaneously incorporate increased amounts of amino acid analogues[5,6]. In addition, the RNA base analogue, 5-fluorouracil induces premature senescence of cultured cells which is preceded by the appearance of altered enzyme[5]. These results have been taken to suggest that the fidelity of protein synthesis decreases, possibly causally, during cellular ageing *in vitro*[5-7].

Cultured fibroblasts derived from subjects with Werner's syndrome, a hereditary disease of accelerated ageing, show a markedly curtailed lifespan compared to normal age-matched controls[3,8,9] Accordingly, one might predict that such cells would show the earlier appearance of abnormal gene products, a higher proportion of abnormal gene products, or a combination of both Since it is difficult to obtain cultures from Werner's subjects before the disorder is clinically fullblown, all strains so far examined have grown poorly from the outset and have lost their replicative capacity quite prematurely Thus, although the first prediction is difficult to test, the second possibility can be analysed Here we demonstrate that a strain of Werner's fibroblasts contains a high defective proportion in 3 different gene products, HL-A2 and HL-A9, two histocompatibility antigens on the cell surface and glucose-6-phosphate dehydrogenase (G-6PD), a cytoplasmic enzyme in the pentose shunt

Cultures were derived in our laboratory following explantation of skin of the anterior forearm as previously described[2] The subject with Werner's syndrome (H McG) was a 56-yr-old female who had already been studied *in vivo*[10] and *in vitro*[3] This strain of Werner's fibroblasts again showed a reduced lifespan so that only five to six subcultures were possible at a 1 8 split ratio, that is, 15–18 mean population doublings (MPD) before total cessation of growth[1-3] Normal controls were capable of 15–25 subcultures (45–75 MPD) in the same conditions G-6PD was assayed for thermolability using crude cell extracts as described by Holliday and Tarrant[5] Briefly, cultures were allowed to grow to confluence and then collected Following rinsing, cells were suspended in 0 05 M Tris buffer, *p*H 8 0, containing 10^{-3} M NADP and sonicated followed by centrifugation for 30 min at 30,000*g* The supernatant was then heated at 60° C Aliquots were removed after various times and immediately placed in an ice bath till assay on a Gilford ultraviolet spectrophotometer HL-A phenotypes were first determined on circulating lymphocytes of the skin fibroblast donors by a microdroplet cytotoxicity technique[11], modified slightly for fibroblasts[12] Quantitative absorption studies were performed using appropriate antisera as previously described[13]

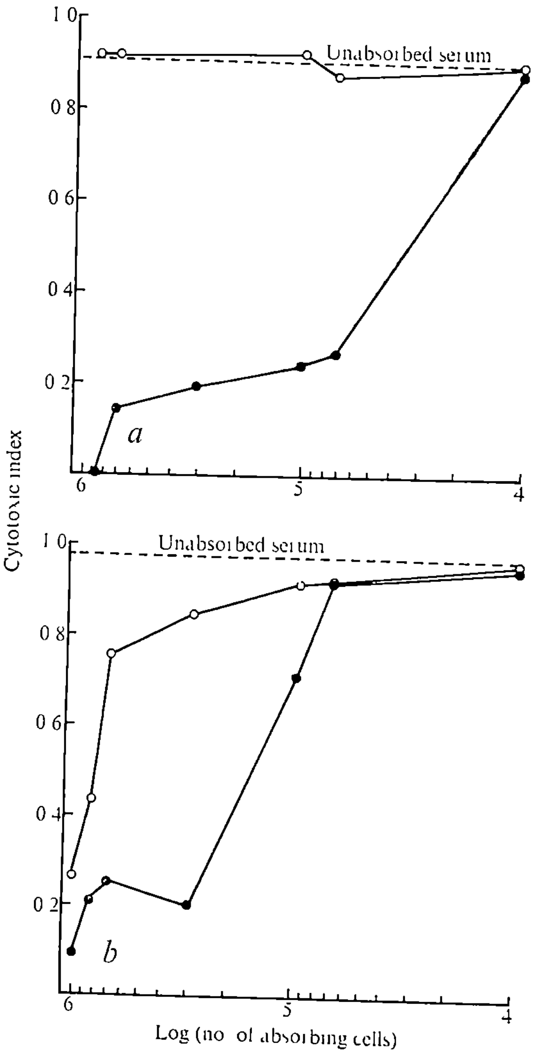

Fig 1 Cytotoxicity of *a* anti-HL-A2 serum and, *b* HL-A9 serum following absorption by fibroblasts from Werner's syndrome and a normal 36-y-old male (HL-A2,9,7,12) Fibroblasts at the concentrations indicated were suspended in 10μl of antiserum and incubated for 40 min with mixing followed by centrifugation and testing of the supernatant against appropriate target lymphocytes Werner's cells were at 15 and 12 MPD (total capacity 18) in *a* and *b*, respectively Normal cells were at 21 and 36 MPD (total capacity 60) ○, Werner's syndrome, ●, normal

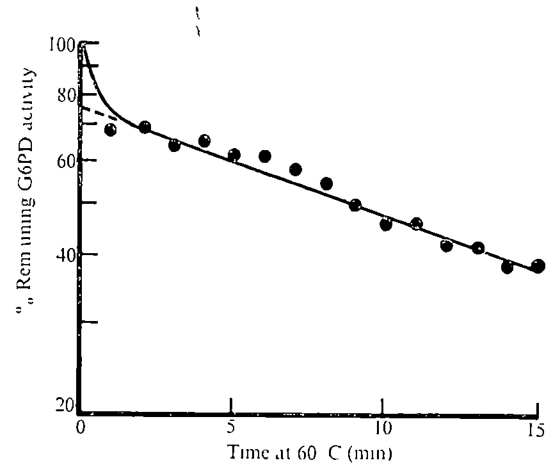

Fig 2 Heat inactivation of glucose-6-phosphate dehydrogenase in extracts of skin fibroblasts derived from a patient with Werner's syndrome Cells were at 12 MPD

The HL-A phenotype of the patient with Werner's syndrome, as determined on her peripheral blood lymphocytes, was HL-A2, 9, 5, W15 All four antigens were detected on cultured fibroblasts by the cytotoxicity method but the results became erratic following the first subculture The results of quantitative absorption studies for two antigens, HL-A2 and HL-A9, are given in Fig 1 The expression of HL-A2 (Fig 1*a*) was markedly reduced on fibroblasts from Werner s syndrome compared to the normal control HL-A9 expression was also reduced (Fig 1*b*) but not as markedly as that of HL-A2 Taking the 50% level of cytotoxicity as a reference point, the quantitative expression of antigens can be calculated[13,14] Thus, HL-A2 expression was reduced more than 50-fold in Werner's fibroblasts compared to the normal control while HL-A9 expression was reduced about 4 5-fold The expression of each antigen in normal strains from donors aged 27–76 yr varies less than twofold throughout the culture lifespan as we and others had described previously[15,16]

Figure 2 indicates that extracts of Werner's fibroblasts had 24% heat-labile G-6PD On two other occasions at similar MPD levels, Werner's cells had 14% and 22% heat-labile G-6PD In contrast, early-passage cultures from normal controls aged 28–76 yr averaged 1 0% heat-labile G-6PD (range 0–4% in five strains tested 17 times) while late-passage controls averaged 4 3% (range 0–10% in four strains tested 17 times) An equal part mixture of extracts from Werner's cells (24% heat-labile) and normal cells (0% heat-labile) gave 9% heat-labile G-6PD, close to the predicted value of 12% This suggests that proteolytic or other detrimental factors are not responsible for the greater degree of heat-labile G-6PD in Werner's cells and conversely, that a deficiency of stabilising factors is probably not involved

The data indicate that three diverse gene products, HL-A2 and HL-A9, two surface-related antigens which are coded for by autosomal genes[17], and G-6PD, a cytoplasmic enzyme which is genetically X linked[18], are markedly abnormal in cultured cells from Werner's syndrome Virtually identical results on G-6PD have recently been found on a different strain of Werner's fibroblasts by Holliday *et al*[9], although the differences between early and late-passage normal cells in this report are much less than previously observed by Holliday and Tarrant in MRC-5, a strain of male foetal lung fibroblasts[5] We recognise that controversy exists regarding the composition of HL-A antigens[19-21] Indeed, if these molecules turn out to be glycoproteins[22] it is possible that their expression depends on glycosyl transferases as well as the protein component coded for by HL-A genes Thus, the altered reactivity with antiserum of HL-A2 and HLA-9 in Werner's fibroblasts may reflect aberrations in more than a single gene product But this does not affect the conclusions and it seems likely, therefore, that these cells have a widespread defect in cellular proteins which is more severe in extent than in ageing normal cultures In other studies, we have found similar disturbances in HL-A antigen expression[13,15] and heat lability of three different cytoplasmic enzymes in fibroblasts derived from subjects with progeria, another disease of premature ageing (S G , unpublished) Additionally, a fraction of clonal subpopulations within normal mass cultures may also develop altered HL-A expression during ageing *in vitro*[12,15]

Although no proof exists that accumulation of defective proteins is the cause rather than the result of cellular ageing[7], our evidence strengthens the concept that the rate of fibroblast ageing *in vitro* is directly related to the rate of ageing *in vivo* Nevertheless, information is still needed to explain how a diversity of protein defects in ageing cells predisposes individuals with Werner's syndrome to diabetes mellitus, severe atherosclerosis and neoplasia[10], the same diseases that increase exponentially during normal biological ageing[4,15,23,24]

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Effects of prostaglandin E_2 on human platelet adenyl cyclase and aggregation

The ability of prostaglandins to affect platelet function has been described in many laboratories[1,2]. The most potent of these, prostaglandin E_1 (PGE_1), has been found to inhibit platelet aggregation and stimulate platelet adenyl cyclase The effect of prostaglandin E_2 (PGE_2), however, has been either inhibitory or stimulatory, depending both upon the species of animal examined[3] and the concentration of prostaglandin used[4]

Recent demonstrations of the ability of platelets to produce and secrete prostaglandins[5,6] have stimulated new interest in the effects of the different prostaglandins on platelet activity

The present investigation was designed to re-examine the effect of PGE_2 on human platelets, using concentrations of prostaglandin comparable with the levels that platelets produce and secrete[5,6] It was found that PGE_2 stimulated platelet adenyl cyclase and inhibited both ADP- and collagen-induced platelet aggregation

Blood was collected into sodium citrate (0 38%) from normal human donors who had not ingested aspirin for a minimum of 14 d Blood or platelets contacted only siliconised glass or metal surfaces, or non-wettable plastic The platelet-rich plasma (PRP) was collected after centrifugation at 150*g* and maintained at room temperature Aggregation was followed by the turbidimetric procedure of Born[7], measuring absorbance decrease at 600 nm at 37° C in either a Gilford 2000 or Unicam SP800 spectrophotometer with magnetic stirring (1,000 r p m) Varying concentrations of either PGE_1 or PGE_2 (Ono Pharmaceuticals) dissolved in dimethylformamide (DMF) (10 µl) were added to the PRP (2 7 ml) and incubated without stirring at 37° C for 10 min At time zero, inducers were added and stirring commenced Appropriate controls preceded and followed each PG aggregation Inducers were ADP (Sigma), 10 µl, in aqueous solution (final concentration, $0\ 9 \times 10^{-4}$ to $1\ 8 \times 10^{-4}$ M) and collagen, 50 µl, prepared according to Hirsch, *et al*[8]

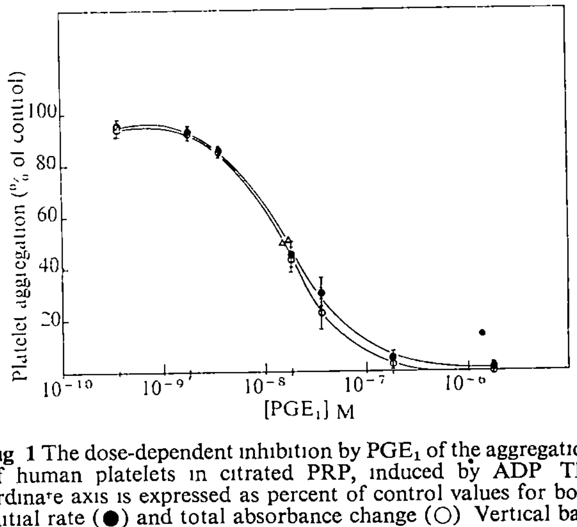

Fig 1 The dose-dependent inhibition by PGE_1 of the aggregation of human platelets in citrated PRP, induced by ADP The ordinate axis is expressed as percent of control values for both initial rate (●) and total absorbance change (○) Vertical bars show standard error of the mean (s e m), 5–8 replications per point The ID_{50} concentration is marked on each curve (△) For other details, see text

Adenyl cyclase studies utilised platelets obtained from EDTA–anticoagulated blood by the method described by Haslam[9] The washed platelets, resuspended in 10 mM Tris (pH 7 5 at 20° C), were kept intact at 4° C until used Cyclase activity was determined in triplicate by measuring conversion of α–^{32}P–ATP to ^{32}P–cyclic AMP Added ^{3}H–cyclic AMP was used as an index of recovery Platelets were used without being lysed Lower levels of cyclic AMP were obtained in platelets lysed either by sonication or freeze–thawing

The assay mixture contained the following components in a final volume of 1 0 ml α–^{32}P–ATP (New England Nuclear), 6 mM (0 5 µCi), phosphoenolpyruvate (Sigma), 0 1 mM, pyruvate kinase (Sigma), 1 0 unit, magnesium chloride, 9 mM, theophylline (Sigma), 10 mM, cyclic AMP (Sigma), 2 0 mmol, potassium chloride, 5 5 mM, Tris buffer, 40 mM, pH 7 5 (20° C), approximately 10^8 washed human platelets

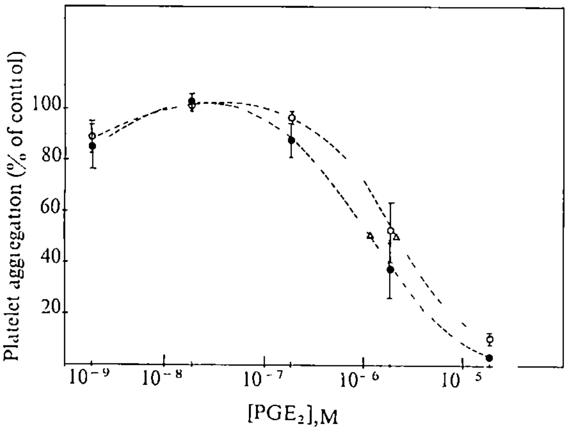

Fig 2 The dose-dependent inhibition by PGE$_2$ of the aggregation of human platelets in citrated PRP, induced by ADP, 8–12 replications per point The ordinate axis, symbols and details are as in Fig 1

As in the aggregation work, prostaglandins were added in DMF, with appropriate blanks and controls Methodology used by one of us (M D) differed slightly and was described previously[10]

After incubation at 37 °C for 15 min, the reaction was stopped by adding 0 1 µCi of ^{3}H–cyclic AMP (50 µl) to each tube followed by heating for 5 min in boiling water The cyclic AMP isolated by the methods described by White[11] and Ramachandran[12] was counted for both ^{3}H and ^{32}P

PGE$_1$ inhibited the ADP-induced aggregation of citrated PRP in the expected dose response relation over a concentration range of $3\ 7\times10^{-10}$ to $1\ 8\times10^{-6}$ (Fig 1) This was evident both when the inhibition was measured as decrease in initial absorbance rate and when it was measured as total absorbance change The correlation between the two measured parameters was excellent with an ID$_{50}$ of $1\ 6\times10^{-8}$ to $1\ 7\times10^{-8}$ M

The effect of PGE$_2$ on ADP-induced platelet aggregation in citrated PRP was similar to that of PGE$_1$ Thus, over a concentration range of $1\ 8\times10^{-9}$ to $1\ 8\times10^{-5}$ M, only inhibition was observed in both the initial rate (ID$_{50}$ = 5×10^{-6} M) and total absorbance change (ID$_{50}$ = 1×10^{-5} M) parameters (Fig 2) PGE$_2$ was therefore only 1/500 to 1/250 as potent as PGE$_1$ in inhibiting the ADP-induced aggregation of citrated PRP Stimulation by PGE$_2$ alone or augmentation of ADP-induced aggregation by PGE$_2$ was never seen

The collagen-induced aggregation in the same system was also inhibited by PGE$_1$ (Fig 3) over the same concentration range ID$_{50}$, measured by initial rate inhibition, was $1\ 2\times10^{-6}$ M • For total absorbance change, the ID$_{50}$ was $2\ 1\times10^{-6}$ M Although it seems that greater inhibition was obtained at the lowest PGE$_2$ concentration, than at a tenfold higher concentration, these data were not statistically different from control values

Platelet adenyl cyclase was stimulated by both PGE$_1$ ($5\ 0\times10^{-9}$ to $5\ 0\times10^{-6}$ M) and PGE$_2$ ($1\ 0\times10^{-7}$ to $2\ 0\times10^{-4}$ M) as shown in Fig 4 Both compounds displayed only stimulation above basal levels, even at the lowest concentrations, in classic dose-response relationships The ED$_{50}$ for PGE$_1$ was 5×10^{-8} M and for PGE$_2$ was 7×10^{-6} M Thus, about 140 times more PGE$_2$ was required to equal a given PGE$_1$ level of stimulation Although absolute amounts of adenyl cyclase activity varied among platelet samples, the ED$_{50}$ values obtained for both PGE$_1$ and PGE$_2$ were consistent and reproducible These effects were obtained whether the platelets were prepared as described here or by a previously published method[13]

Several studies using PGF$_{2\alpha}$ (Fig 4) were also performed Though stimulation was minimal, a decrease of adenyl cyclase activity below basal levels was never seen

In the platelet aggregation studies, the effects of PGE$_1$ and PGE$_2$ were inhibitory only, as measured by two parameters over a wide concentration range Contrary to published results[3,14], no stimulation of either ADP- or collagen-induced aggregation at low PGE$_2$ concentrations was observed The difference between this and previous studies may be in the reproducibility of aggregation results In citrated PRP, even a series of control experiments on the same platelet sample will yield results that vary with time and other factors we have not yet identified, under presumably identical conditions To circumvent this, the values obtained for each prostaglandin treatment were compared with control values obtained both before and after the PGE inhibition experiment Many experiments were performed at each inhibition concentration with many different platelet samples In the ADP-induced aggregation studies inhibited by PGE$_2$ below 2×10^{-6} M, over 60 separate experiments, exclusive of controls were performed with platelets from 17 different donors The collagen-induced experiments were replicated fewer times, hence the larger standard errors (Fig 3) Even in these experiments though, inhibition was obvious

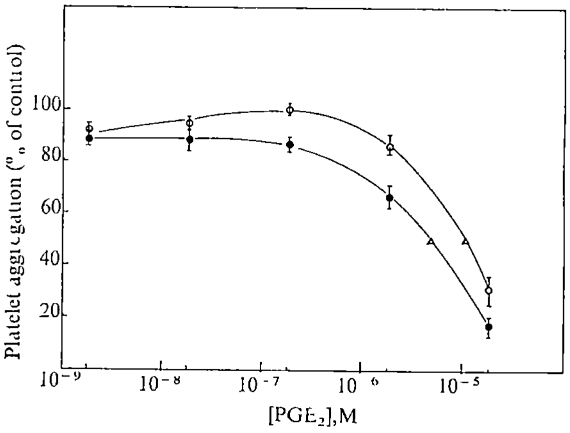

Fig 3 The dose-dependent inhibition by PGE$_2$ of the aggregation of human platelets in citrated PRP, induced by collagen, 4 replications per point The ordinate axis, symbols and details are as in Fig 1

The stimulation of platelet adenyl cyclase activity by PGE$_1$ at various concentrations agrees with previously reported data[10,13] PGE$_2$ similarly elicited no response other than stimulation of the platelet adenyl cyclase This is contrary to previous published reports[4] in which PGE$_2$ at $2\ 8\times10^{-7}$ M (0 1 µg ml^{-1}) was found to inhibit adenyl cyclase activity In the earlier work, emphasis was given to the finding that only at low concentrations did PGE$_2$ inhibit adenyl cyclase activity Concentrations which we found stimulatory, however, were the same as those previously reported as inhibitory The reason for this discrepancy is not clear

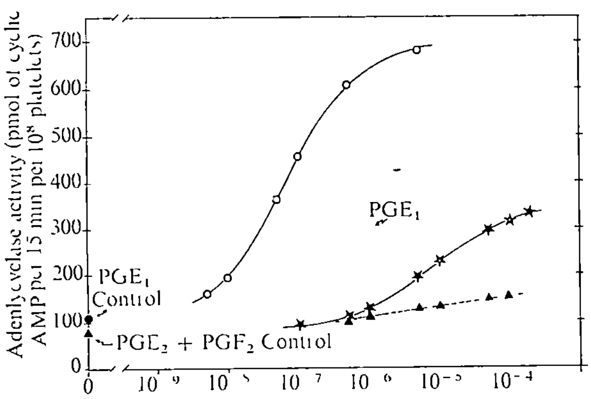

Fig 4 The effects of PGE_1 (○), PGE_2 (★), and $PGF_{2\alpha}$ (▲) on washed human platelet adenyl cyclase The PGE_1 data were obtained using different platelets from the PGE_2 and $PGF_{2\alpha}$ data For reference, a single concentration of PGE_1 (△) was assayed in the PGE_2–$PGF_{2\alpha}$ platelets In other experiments not reported, PGE_2 and $PGF_{2\alpha}$ were studied at concentrations as low as 10^{-8} M with results similar to PGE, (M D) At PGE_1 concentrations above 10^{-5} M, a decreased output of cyclic AMP has been observed (unpublished data) It has not been possible to show this effect with PGE_2, possibly because of difficulties in getting high enough concentrations in solution

Recent studies have suggested the existence of a regulatory or feedback mechanism of platelet function in which prostaglandins, produced and secreted in response to thrombin, may stimulate platelet adenyl cyclase to produce cyclic AMP and thereby modulate the release and aggregation processes (refs 6, 15 and M D, unpublished data) Our findings that both PGE_2 and PGE_1 stimulate adenyl cyclase supports this hypothesis, especially as we found that presumably physiological levels of PGE_2 (refs 5, 6) were stimulatory

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Delayed fertilisation in senescent golden hamsters

REPRODUCTIVE senescence in the female golden hamster occurs after 14 months of age and is characterised by a significant decline in the production of offspring[1] Recent studies in our laboratory concerned with the cause(s) of this decline have revealed large numbers of non-viable ova in aged hamsters before implantation[2] and a developmental delay of approximately 12 h in preimplantation and implantation stages of embryos from senescent hamsters when compared with young females at the same hour of pregnancy[3,4] Since the elapsed time for the union of gametes is important for normal fertilisation and development we examined ova for the onset of fertilisation in young and aged hamsters

One hundred and twenty young virgin (3–5 months old) and 170 multiparous senescent (14–17 months old) hamsters, cycling regularly, were mated with young males (3–6 months old) at the onset of behavioural oestrus Ovulation was considered to occur 8 h from the onset of oestrus[5] and all developmental stages are based upon this assumption All animals were from the Oregon colony, except for 72 senescent females from commercial breeders Ova collected from 1 h through 12 h and at 1, 1½, 2, 2½, 3 and 3½ d intervals, were either mounted *in toto* and examined with differential interference contrast microscopy (Nomarski optics) or compressed, fixed and stained with lacmoid

Most of the ova from senescent females showed a 2 to 5 h delay in fertilisation compared with young females (Table 1) The delay was not merely an inbred characteristic of females from the Oregon colony, since aged females from two commercial animal farms demonstrated the same trait

Although there seemed to be a short delay in the completion of ovulation in the senescent hamster 1 h after ovulation (10 5±1 12 compared with 13 8±0 91 for young females), there was no statistical difference in the number of ova at 2 h (12 8±2 25) compared with ova from young hamsters 1 h after ovulation The total number of ova recovered from young and senescent females was similar throughout the first 12 h (young 13 2±0 33, senescent 12 1±0 38), but a real difference was apparent when the number of normal-appearing ova was determined (young 12 6±0 40, senescent 10 9±0 30), large numbers of non-viable ova were found in the aged hamsters Some ova were apparently defective at the onset of ovulation since they were in an advanced state of deterioration at the time fertilisation normally occurs in young females Other fertile ova of normal appearance stained with lacmoid revealed anomalies commonly associated with a delay in fertilisation, such as fragmented pronuclei (6 ova), dispermy (3 ova), trispermy (1 ovum) and androgenesis (1 ovum) On examining zygotes

Table 1 Normal ova and their stage of fertilisation from young and senescent hamsters

Hours after ovulation	Percentage					
	Ova penetrated by spermatozoa		Ova with second polar body		Ova with second* polar body and male and female pronuclei	
	Y	S	Y	S	Y	S
2	—	0 0	—	0 0	—	0 0
3	44 2	16 7	29 5	8 3	0 0	0 0
4	90 1	37 3	77 5	25 3	0 0	0 0
5	100	40 9	94 3	29 6	75 0	1 6
6	—	51 5	—	36 6	—	20 9
7	—	78 0	—	65 9	—	41 5
8	100	94 4	100	78 9	100	73 7
9	—	80 3	—	78 9	—	78 9
10	100	72 3	100	69 1	100	69 1
11	84 5	94 1	84 5	91 2	84 5	91 2
12	96 1	100	96 1	98 4	95 4	91 1

Y, Young
S, Senescent
* Ova stained with lacmoid

from the uterus, we found that approximately 40% of the ova from senescent females were incapable of implantation so that preimplantation loss seems a major factor. Unfertilised ova made up a small percentage of the non-viable ova (1.4% compared with 1.3% for young females), therefore, it is conceivable that the delay in fertilisation was sufficient to have caused some embryonic loss.

Researchers have shown that delaying fertilisation in young golden hamsters by artificially inseminating[6] or naturally mating females[7] 3 h after ovulation, results in considerable embryonic loss (14% to 61%). In both studies capacitation of the spermatozoa would have required between 2 and 4 h (ref. 8), therefore, the ova were between 5 and 7 h old before fertilisation. In our study many ova from aged hamsters were not fertilised until 5–8 h after ovulation.

Spermatozoa may be slowed in their ascent through the aged reproductive tract, although oviducts compared from several young and senescent females allowed to breed for 1 h from the beginning of coitus revealed no difference in the approximate number or location of spermatozoa. No spermatozoa were detectable, however, in ampullae of oviducts of either group. A more probable cause for the delay is prolonged capacitation. This may explain why some ova from senescent hamsters in the present study are fertilised at a time when hamster spermatozoa have been shown to be no longer viable[9]. Raised levels of plasma progesterone have recently been reported in some senescent hamsters during oestrus and the first day of pregnancy[10]. This may indicate that the ageing hamster uterus is less capable of binding progesterone, a condition noted *in vitro* with the senescent rabbit uterus[11]. A refractory uterus could be causing a delay in fertilisation in hamsters since subcontraceptive levels of progesterone may delay fertilisation in rabbits[12] either by slowing spermatozoa migration through the reproductive tract and/or by prolonging capacitation[13,14].

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Abnormal potassium conductance associated with genetic muscular dystrophy

DYSTROPHIC diseases not only lead to progressive deterioration of muscle, but alter several properties of the erythrocyte surface[1-4], and permeability of various other membranes[5-7]. Muscular dystrophies are also associated with defects in the nervous system, so that a neurogenic aetiology[8] has been suggested, there being reports of peripheral neural dysfunction[9], increased frequency of mental retardation[10] and defective auditory acuity[11].

Many of these non-muscular (systemic) features of the dystrophic condition are associated with cellular membranes, indeed dystrophic muscle itself has an abnormal membrane composition[12] and greater than normal calcium-linked ATPase activity in the sarcoplasmic reticulum[13]. Moreover, dystrophic muscle exhibits a significantly altered ionic content and abnormally large potassium conductance[14], although these attributes may only reflect the characteristic degeneration of such tissue. I report here that diverse membrane systems in dystrophic animals and humans conduct potassium more rapidly than in the case of normal subjects. This higher conductance could, in principle, account for the disease process.

Table 1 shows passive K+ efflux from erythrocytes from normal subjects and patients with Duchenne muscular dystrophy where the rate of efflux is about five times greater. In other experiments, the cardiac glycoside, ouabain, was without influence on the efflux and addition of the K+-conducting antibiotic, valinomycin, stimulated it. The extent of K+ loss in the presence of valinomycin, suggests that there is no consistent difference between the K+ content in normal and dystrophic erythrocytes.

Table 1 Efflux of potassium from human erythrocytes

Source of cells	No. of subjects	Potassium efflux (nmol per min per 10^8 cells)
Normal subjects	8	21.6±2.4
Duchenne dystrophy patients	7	107.9±7.5

Results are expressed as mean and standard error of the mean. Samples of venous blood were drawn into a heparinised tube, cells were sedimented at 1,500*g*, resuspended in isotonic saline, washed twice and finally resuspended in about one-tenth of the original volume of saline. All operations were carried out at 0° C. Potassium efflux was measured at 20° C with a Corning model 476132 potassium electrode which exhibited an 80-fold selectivity of potassium as compared with sodium. The medium contained 360 mM sucrose, 15 mM NaCl and 0.1 mM KCl in a volume of 10 ml to which was added 0.4 ml of an erythrocyte suspension containing approximately 10^9 cells per ml.

Figure 1 shows light scattering changes after addition of potassium acetate to suspensions of mouse liver (*a*) and brain (*b*) mitochondria. Normal mitochondria from both organs are relatively impermeable to K+ whereas those from dystrophic animals took up a significant amount. I have observed a similar increase in K+ conductance with liver mitochondria from a second strain of dystrophic mice (129ReJ-dy) and from nutritionally dystrophic rats maintained on a vitamin E-deficient diet. This last observation suggests that membrane dysfunction may be the basis for similarity between genetic and nutritional dystrophy. Addition of Na+ and Li+ acetate does not result in significant changes in light scattering by mitochondria from either normal or dystrophic animals. In dystrophic mitochondria, the uptake of K+ is probably energy-linked, as subsequent addition of 2 µM carbonylcyanide *p*-trifluoromethoxyphenylhydrazone, an uncoupler of oxidative phosphorylation, returns the absorbance to its initial value.

Thus there is greater than normal conductance of K+ across erythrocyte, liver mitochondrial and brain mitochondrial membranes in the dystrophic state. Since none of these tissues seem to degenerate as muscle does, the genetic defect in muscular dystrophy may give rise to membranes, throughout the organism, which are leaky to K+, and muscle deterioration may be a consequence of that leak. This may occur through defective excitation-coupling where rapid K+ movement might serve to collapse charge dislocation required for a cycle of membrane depolarisation–repolarisation. In this connection, it is interesting that the T tubular system, a major locus for K+ transport to the muscle interior, has been shown to be structurally altered in muscular dystrophy[15].

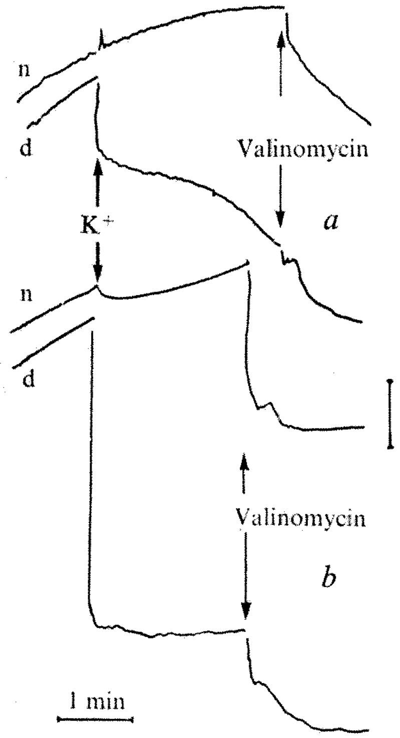

Fig. 1 Light scattering changes caused by addition of potassium acetate to mitochondria. *a*, Liver mitochondria; *b*, brain mitochondria. n denotes mitochondria isolated from normal mice (Bar Harbor strain C57BL/6J); d denotes mitochondria isolated from genetically dystrophic mice (Bar Harbor strain C57BL/6J-dy^{2}J). K$^+$ denotes addition of 4.0 µeq potassium acetate; valinomycin denotes that of 0.5 µg of the potassium ionophore, valinomycin. Light scattering was measured as the change in absorbance at 540 nm at 20° C, a downward deflection corresponding to swelling (uptake). Liver mitochondria were isolated in 250 mM sucrose while brain mitochondria were isolated in a medium containing 300 mM mannitol and 0.1 mM ethylenediamine tetra-acetate and adjusted to *p*H 7.4. The light scattering measurements were carried out in 3.0 ml of the appropriate isolation medium supplemented with 5.0 mM bicine, *p*H 7.4. The vertical bar in the figure indicates an absorbancy difference of 0.003 units.

Finally, the K$^+$ leak as an explanation for the origin of muscular dystrophy is consistent with the observation that hypokalaemic periodic paralysis, which clearly reflects anomalous K$^+$ distribution across membranes, frequently occurs in families with a history of muscular dystrophy[14]. It should also be viewed in light of the report that serum creatine phosphokinase activity, a measure of muscle deterioration, decreases in dystrophic patients after administration of lithium gluconate[16], which is a non-competitive inhibitor of K$^+$ translocation in various biological systems[17].

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Possible role of zonula occludens of the myelin sheath in demyelinating conditions

AUTOIMMUNE mechanisms may play a major role in several human demyelinating diseases[1]. Myelin contains components, principally a basic class of protein, which, when injected systemically with an adjuvant, elicit in the experimental animal a demyelinating encephalomyelitis or neuritis with perivascular infiltration of lymphocytes and inflammatory cells[1,2]. A satisfactory hypothesis is that the potential self-antigens are secluded from the immune systems in the normal living animal[3]. Evidence for such a specific compartmentation of myelin components, however, has been lacking up to now. This has led several authors to suggest alternative explanations[1,4,5]. Here we demonstrate the structural basis for a seclusion mechanism within both peripheral and central myelin sheaths in different animal species.

A continuous tight junction, or zonula occludens, is present at the borders of each myelin segment. This junction separates an intramyelinic extracellular space from the intercellular compartment of the nervous tissue. In addition, the junctional strand separates the internal portion of the glial membrane exposed to the tissue space from the internal portion of the glial membrane exposed to the intramyelinic extracellular space and constituting the compact myelin.

Blocks of peripheral nerve and of various regions of the CNS, including cerebellum, medulla oblongata, optic nerve and forebrain, were obtained from turtles, chickens and rats perfused with fixatives containing aldehydes, or with ferrocyanide-reduced osmium tetroxide[6], and processed for thin sectioning and freeze-fracturing according to routine methods[7,8]. In a more extensive article[9], we have shown that thin sectioning of blocks fixed with ferrocyanide–osmium and freeze-fracturing confirm the existence, already partly established in the literature[10], of an extracellular space within the myelin sheath, not only in the peripheral but also in the central nervous system. This extracellular space is situated between the paired minor dense lines (that is, the external leaflets of the myelin membrane) as predicted from X-ray diffraction studies[11].

In sections, the tight junctions of the myelin sheath appear as spots of membrane fusion (Fig. 1 *a*, *b*). In peripheral sheaths they occur at the internal and external

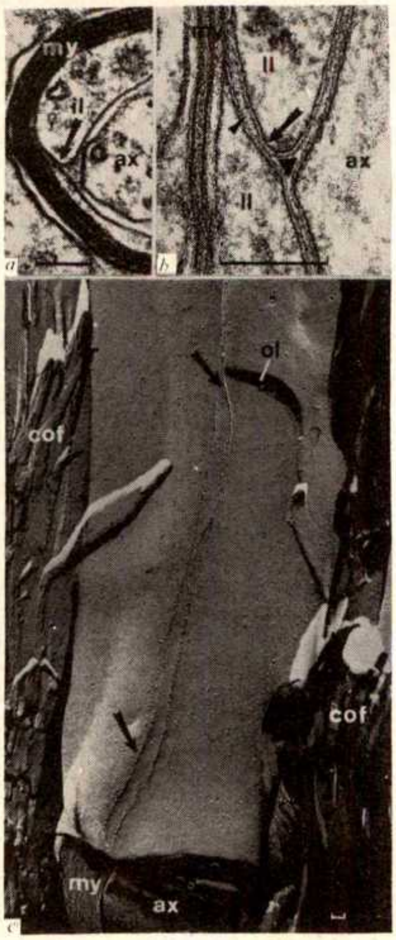

Fig. 1 *a*, Electronmicrograph of a central myelinated fibre showing the tight junction (arrow) of the internal mesaxon, that is, the site where the inner loop (il) joins the compact myelin (my). The axon is labelled ax. *b*, a central myelinated fibre showing the tight junction (arrow) between two paranodal loops (ll) near the Ranvier node. The paranodal loops represent the winding lateral loop passing repeatedly through the plane of section (compare Fig. 2 *d* and *e*). The arrowhead indicates the intramyelinic extracellular space, and the triangle the periaxonal space. *c*, Electronmicrograph of a replica of a freeze-fractured peripheral myelinated fibre. The arrows indicate tight junctional strands cleaved with the A face of the Schwann cell membrane at the external mesaxon (that is, the site where the outer loop terminates the myelin sheath). The cross-fractured outer loop is labelled ol; cof indicates collagen fibres. The scales in the photographs represent 0.1 μm.

'mesaxons' and between adjoining 'paranodal loops' (terminology explained in the figure legends). In central sheaths the tight junctions occur at the mesaxon (internal), between adjoining paranodal loops[32], and at the site where the outer loop (or outer tongue) joins the compact myelin. The latter site is considered, therefore, as the equivalent of the external mesaxon of peripheral sheaths.

Freeze-fracturing confirms these findings. This technique splits cell membranes, thus producing two artificial fracture surfaces, referred to as A (cytoplasmic) and B (external) 'faces', which complement each other in the hydrophobic interior of the membrane[12]. As for the tight junctions in other tissues[15], strands (Fig. 1 *c*) and furrows are seen on the A and B faces, respectively, at all sites where spots of membrane fusion were observed in thin sections. In addition, the replicas of freeze-fractured myelin sheaths give a more complete tridimensional picture of this tight junctional system, demonstrating that there is a continuous zonula occludens extending from the outer to the lateral and inner loops. This is shown schematically in Fig. 2 *d*, *e*, where the myelin sheaths have been represented as 'unwrapped'. The outer, lateral and inner loops represent portions of the continuous marginal belt of the glial sheet which contains a rim of cytoplasm.

Tight junctions are also present between the loops of the circumferential Schmidt-Lanterman incisures and between the longitudinal protoplasmic islands. These are channels of cytoplasmic retention which occur in some sheaths and which interrupt the compact myelin. The strand(s) of these junctions are continuous with the junctional strands of the mesaxons[9]. There is, therefore, no interruption of the zonula occludens at these sites.

It has been demonstrated in several organs that the zonula occludens, although partly permeable to water and small solutes, constitutes an efficient barrier to the extracellular diffusion of large molecules[15]. While the joining of three neighbouring cells in epithelia might represent the site of small leaks, the zonula occludens of the axonal sheath is formed by a single cell, and the barrier might turn out to be particularly effective. Lanthanum has been observed occasionally to penetrate into the myelin sheath[13,14], but this might be an artefact. Tracers of large molecular weight do not enter the intramyelinic extracellular space[16–18]. The existence of a zonula occludens was not considered in the experiments with tracers. We suggest here that it is the zonula occludens at the inner, outer and lateral borders of the sheets forming the myelin tube that makes the myelinic extracellular space a secluded compartment, thus sequestering some myelin components. According to various authors, the basic encephalitogenic protein is located, at least in

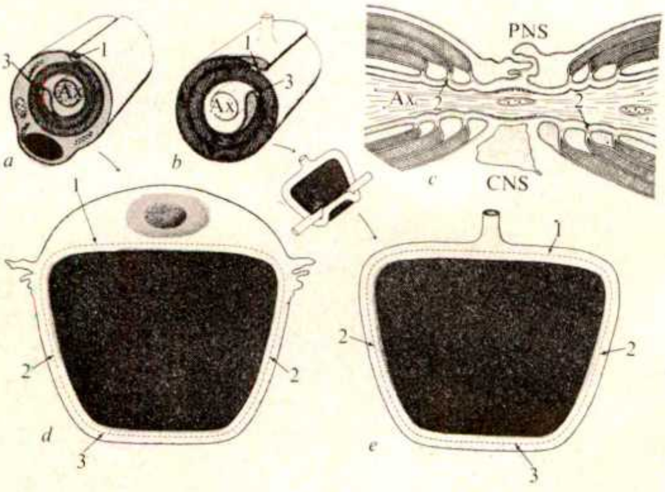

Fig. 2 Diagrammatic representation of peripheral and central myelin sheaths. The illustrations *a*, *b* and *c* are quasi-realistic. In *d* and *e* the axon (Ax) has not been represented, and the complete sheath of one internode has been figuratively unrolled as shown in the unlabelled inset. Portions of peripheral (*a*) and central (*b*) myelin sheaths in tridimensional view. The intramyelinic extracellular space (cross-hatching), comprised between the paired minor dense lines, is emphatically exaggerated. The arrows point to the tight junctions separating this space from the extracellular space within the tissue (arrow 1) and the periaxonal space (arrow 3) (also exaggerated). *c*, Schematic view of the Ranvier node and the paranodal region of peripheral (PNS) and central (CNS) myelinated fibres in longitudinal section. The arrows (labelled 2) point to tight junctions of the spirally winding lateral loop. The periaxonal space is communicating with the extracellular space through the channels of the paranodal axoglial junction. This junction, corresponding to the scalloped portion of the axolemma, has not been represented here and is described in refs 9, 31–33. Peripheral (*d*) and central (*e*) myelin sheaths represented as unwrapped. Arrows 1, 2 and 3 indicate the outer, lateral and inner cytoplasmic loops, respectively. The dashed line shows the extent of the zonula occludens encircling the area of compact myelin (shaded area). For the sake of simplicity, this dashed line corresponds to only one-half of the complete junctional specialisation and is drawn on the middle rather than on the side of the lateral loops and on the axonal aspect of the inner loop rather than on the opposite aspect.

part, at the minor dense line[19,20], that is, it borders on the intramyelinic extracellular space Following damage to the myelinic tight junctions, components of this normally secluded compartment may leak out, thus provoking phagocytic and ultimately autoimmune reactions

The presumptive role of the zonula occludens suggested above is probably a corollary of other functions which might be first, the maintenance of the homeostasis of the extracellular material providing interlamellar adhesion in the compact myelin, and, second, the separation of two different intramembranous regions of the same cell, that is, the glial outer membrane and the membrane of compact myelin

There are experimental conditions in which degeneration of the myelin sheath and its removal by phagocytes is produced without concomitant damage to the axon[21–25] but without proven involvement of the immune systems We wonder whether or not the effect of the experimental damage involves the strand(s) of the zonula occludens also in these cases

There are other organs and tissues which contain potential self-antigens We are particularly struck by certain analogies between the spermatic system and the myelin Sperm contain antigens which can elicit formation of serum sperm agglutinins and immobilisins as well as cellular infiltrates in the testis[26,27] It has been shown[28] that in the spermatic pathways there is a luminal barrier to extracellular diffusion constituted by epithelial zonulae occludentes In addition, at the seminiferous tubules, where sperm are formed, there is a blood–testis barrier[29] Thus, the sperm are secluded from the immune systems[30] The compartmentation produced by the myelinic zonula occludens and by the blood–brain barrier[16] might have, to some extent, functional implications analogous to the compartmentation in the spermatic system

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Light-evoked release of glycine from the retina

GLYCINE is one of the amino acids now regarded as putative neurotransmitters It is present in high concentration in the retina[1,2] and an active high affinity uptake system into a type of amacrine cells has been demonstrated[3,4] Moreover, glycine has inhibitory effects in the retina[5,6]

It has been demonstrated in other parts of the central nervous system that amino acids can be released by electrical stimulation or ionic shock[7–14] It has also been shown, however, that such crude methods of stimulation will release glycine (and some other amino acids) from tissues where it is unlikely to be a neurotransmitter[15] We have therefore studied the release of glycine from the retina, which can easily and effectively be stimulated by light flashes, either *in vivo* or *in vitro*

The anterior segment and the vitreous were carefully removed from the eyes of Nembutal anaesthetised cats, exposing the retinae for the *in vivo* studies Rabbit retinae were carefully excised with the choroid and maintained in a small organ bath at 37° C for the *in vitro* experiments The retinae were exposed to tritiated glycine by superfusing them at a rate of 1 ml min^{-1} for 10 min with a salt solution containing 40 μCi glycine-2-^{3}H or by injecting 10 μCi intravitreally (rabbits) 2 h before excising the retina Initially 0 9% NaCl was used in the cats, but later, Krebs–Ringer–bicarbonate was used with equal results In the rabbit, the solution described by Ames[16] was used The retinae were washed (1 ml min^{-1}) in darkness with the salt solution for 20–40 min and then exposed to one or two 10 min periods of light flashes (2 H$_2$) 10 min apart From the washing and onwards, the salt solution contained 0 1 mg ml^{-1} glycine The radioactivity of the superfusate was monitored by liquid scintillation spectrometry either directly or in the glycine spot in thin layer silica gel chromatography (solvent *n*-propanol water, 70 30 v/v)

The site of uptake of radioactivity into retinae was checked by autoradiography in two experiments and was found to be almost exclusively in a type of amacrine cells, in good agreement with previous results[3,4,17] More than 95% of the radioactivity in the retinae or in the superfusate moved like glycine in the chromatograms.

Thirty-three experiments were performed on rabbit retinae. The radioactivity of the superfusates was usually decreasing throughout the experiments, on the average by a factor 0·79 (s.e.m. = ±0·029) during the 5 min preceding the light stimulation. During this, however, the radioactivity increased by a factor 1·30 (s.e.m. = ±0·103), measured from the start of the stimulation to the peak of the release (which appeared after 3–7 min). The difference between the two factors is highly significant ($P < 0.001$ in the Student's two-tailed *t*-test). One experiment is shown in Fig. 1. In this particular one and also in another, small aliquots of the superfusate fractions were chromatographed, and the radioactivity of the glycine spots were measured. A peak was seen, exactly matching that of the crude superfusate fractions (see Fig. 1). Autoradiograms of the thin layer chromatograms did not reveal any other radioactive spot.

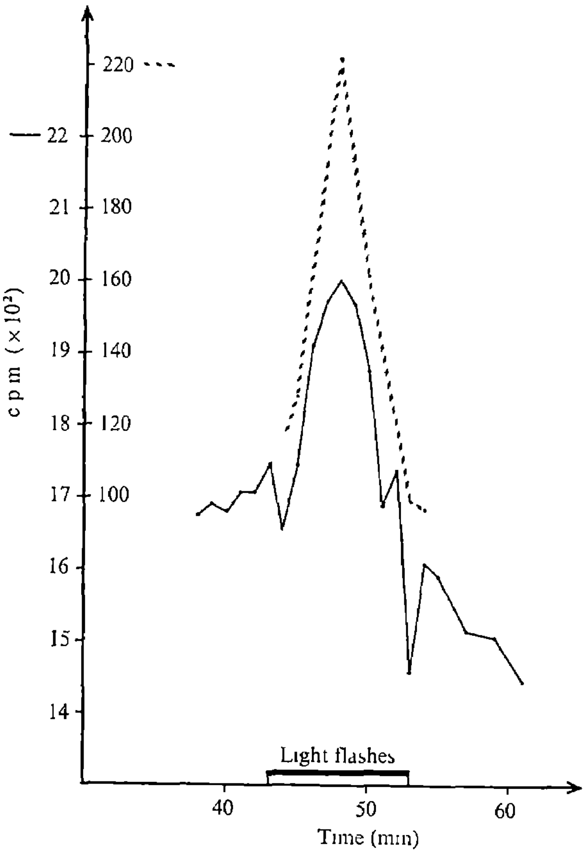

Fig. 1 Efflux of radioactivity in a single experiment from light stimulated rabbit retina *in vitro* after incubation with ^{3}H-glycine ——, radioactivity of the crude effluent (scale on the left hand side of the ordinate), - - -, radioactivity in the glycine spot from silica gel thin layer chromatograms of the effluent (scale on the right-hand side of the ordinate). The difference in absolute radioactivity in the two curves is caused chiefly by differences in sample size. The light flashes were given at 2 Hz with a xenon flash tube driven by 0·6 J per flash and located 30 cm from the retina.

The ^{3}H-glycine was given by intraocular injection in 14 of the 33 rabbit experiments, and by incubation in the remaining 19. There was no statistically significant difference between the results of these two subgroups. The increase in the outflow of radioactivity was highly significant ($P < 0.005$) in each of them.

A second stimulation was given in 13 of the rabbit experiments, and resulted in a smaller and less distinct increase of the radioactivity of the superfusate than the first stimulation. During the five minutes immediately before the second stimulation the radioactivity on average decreased by a factor 0·92 (s.e.m. = ±0·042), and during the stimulation it rose on average by a factor 1·15 (s.e.m. = ±0·100) above the starting value. The difference is possibly significant ($P < 0.05$).

In the experiments on anaesthetised cats, the results were very variable, usually for obvious technical reasons such as incomplete removal of the vitreous, bleedings clogging the superfusion cannulas, movements of the animal and so on. In about twenty attempts, five superfusates showed a smooth baseline curve with a clear peak appearing during the light stimulation. On the average, the peak of these five curves reached 2·94 times (s.e.m. = ±0·485) above the level at the beginning of the stimulation, this contrasts with the preceding 5 min, when the radioactivity fell, on average, by a factor of 0·79 (s.e.m. = ±0·093).

Thus, light stimulation can release radioactivity from retinae preloaded with ^{3}H-glycine. The chromatograms of the superfusate or of the whole retina suggest it is glycine that is released. Serine is a precursor and also a metabolite of glycine, however, and does not separate well from it in chromatograms. Therefore, the experiments do not show to what extent the released radioactivity might represent serine. This is not highly neuroactive, however, and is not generally regarded as a putative neurotransmitter. On the other hand, glycine has been shown to fulfil several criteria of a neurotransmitter in the retina: it is present in high concentration[1,2]—although the precise cellular location remains to be established, there is an efficient neuronal uptake mechanism that conceivably inactivates released glycine[3,4], and applied glycine has clear inhibitory effects[5,6].

Our demonstration of light-induced release of radioactivity from retinae preloaded with radioactive glycine further supports that it is a retinal neurotransmitter. It is of particular importance that the release can be achieved both *in vivo* and *in vitro* and with a physiological stimulus, as this suggests that it is the result of normal nerve activity.

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Does the central human retina stretch during accommodation?

Clear evidence that the human retina stretches during accommodation was presented by Moses[1], but his measurements were all made at the anterior margin of the retina, so they did not show how this stretch is spatially distributed. If it is largely confined to the region of the *ora serrata*, its contribution to the perceptual correlates of accommodation will be much less than if the central retina is distorted as well. A perceptual effect involving accommodation-dependent distortions in the visual field has recently been attributed by its discoverers[2] to such a stretching of the central retina, although non-retinal explanations for this effect are also tenable.

It seemed worthwhile to do an experiment in which the possibility that the central human retina stretches during accommodation could be evaluated, and in which the magnitude of any stretch which was found could be assessed.

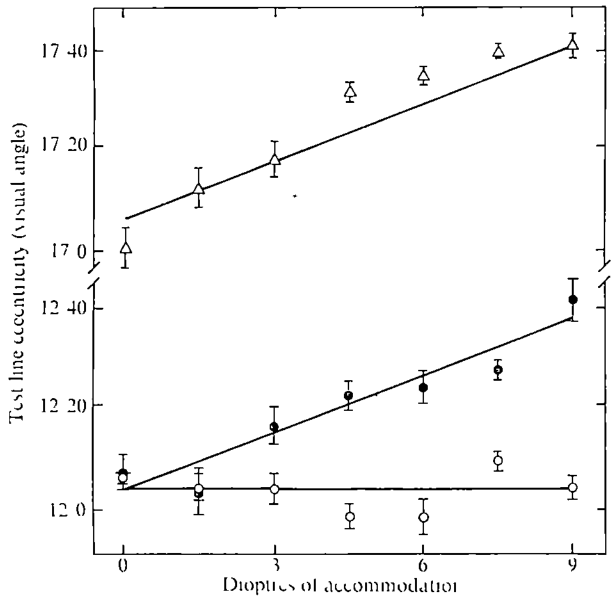

Fig. 1 Test line settings made by the first observer are shown as a function of dioptres of accommodation. Closed filled circles refer to the near edge of the blind spot, the line drawn through them by eye indicating a 4.7% increase in visual angle over the range of accommodation. A line parallel to this has been drawn through the triangles which refer to the far edge of the blind spot. The open circles are control data obtained during cycloplegia: a horizontal line has been drawn through them to indicate that there is no stretching of the retina in these conditions. Error bars show 1 s.e.m. above and below each point, which is the mean of ten determinations. A long test line (2° from top to bottom) and a high retinal illuminance for the background (5.6 log trolands) were used to make the line as conspicuous as possible once it had emerged from the blind spot.

The observer's left eye was patched throughout, and the right provided with a Maxwellian view of a fixation cross and a vertical test line, both seen in silhouette against a background of monochromatic yellow (575 nm) light. The test line was located to the right of the fixation cross, its exact horizontal position was under the observer's control. Because the eye was positioned one focal length behind the Maxwellian lens, the apparatus served as a Badal optometer: that is, movements of the target plane (containing both the fixation cross and the test line) along the optic axis stimulated different degrees of accommodation but did not affect the size of the retinal image.

The observer's task was to fixate the centre of the cross and to keep it in sharp focus, while at the same time moving the test line until it was just visible on the near side of the blind spot (the side closer to the fixation cross). Measurements were made at seven different levels of accommodation (Fig. 1) to plot the distance in visual angle between the fixation cross and the test line. Since the test line marks the near edge of the blind spot, the data imply that the fovea and the optic disk are more widely separated when accommodation is strained than when it is relaxed.

An alternative interpretation of the data would be that threshold criteria changed systematically as a result of accommodative effort, or that the target was focused on the retina more sharply at one level of accommodation than at another. These possibilities were tested, as a class, by measurements analogous to the first set but now localising the far side of the blind spot: if blurring or criterion shifts were involved, the line should have been set closer to the fixation cross as accommodation increased. Just the opposite was found (Fig. 1), thus eliminating these possibilities. The results of these two experiments were confirmed on a second observer.

There remained the chance that some misalignment of the apparatus, rather than a stretching of the retina, produced the effect. To rule out this possibility a control was run with accommodation paralysed with 1% Cyclogyl, but with the target plane moved along the optic axis as before. A smaller aperture stop was now used to reduce the diameter of the pencil of light passing through the pupil centre to less than 0.5 mm, thereby ensuring that the stimuli would always be seen in sharp focus despite the lack of accommodation. The effect found earlier was now clearly absent (Fig. 1).

A final experiment was undertaken to determine how great a contribution to the effect is made by changes in the magnifying or other properties of the crystalline lens. It is known[3] that convergence can bring about accommodation, and it seems likely that in a presbyope, devoid of accommodative ability, convergence would still be accompanied by a central command for accommodation. The lens would not respond to such a command, but if the ciliary muscle contracted, then the retina might be stretched just as in a younger subject.

This possibility was investigated using a 66-yr-old presbyopic observer. A different apparatus (with slightly different stimulus dimensions) was employed so that the observer could be presented with the fixation cross binocularly, while the test line was as usual seen by the right eye only. Natural viewing at a distance of 3 m was used, and the observer moved the test line with a pulley so that it fell on the near margin of the blind spot. While the right eye remained in the primary position throughout, mirrors were introduced in front of the left eye to make it converge a total of 25°. Measurements made both with and without the mirrors showed that the visual angle between the fixation cross and the blind spot increased some 4.6% when convergence was brought into play, this is comparable to the figures obtained during actual accommodation in the initial experiments: 4.7% for one subject (age 28) and 4.4% for the other (age 23). This similarity of results for the presbyopic and non-presbyopic observers suggests that changes in the lens do not play a primary role in producing the perceptual effect under study.

The results of the present study, taken together, argue persuasively that the central region of the human retina stretches substantially—some 4½%—during marked (9 dioptre) accommodation. This is much larger than the figure of 2% stretch, which is obtained by dividing Moses' measured 0.5 mm advance of the *ora serrata* by the distance from the *ora* to the optic disk[4], the reason for this difference remains unknown. The stretching reported here has implications for our understanding of accommodative micropsia[5] and other perceptual phenomena: in particular the stretch (if it is non-uniform) may underlie the spatial distortions discovered by Blank and Enoch[2], as in fact they propose, but this remains to be established. The stretching of the central human retina may also be of interest to those who study the eye from a clinical point of view.

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Acetylcholine as an excitatory neuromuscular transmitter in the stomatogastric system of the lobster

THERE is much physiological evidence that L-glutamate acts as an excitatory transmitter at many arthropod neuromuscular synapses[1-3] The only suggestion that acetylcholine (ACh) has such a function is due to Futamachi[4] He showed that the nerve terminal region innervated by the largest crayfish slow flexor excitatory motor neurone is depolarised by iontophoretic applications of ACh, but not L-glutamate I now have biochemical and physiological evidence that ACh is the neurotransmitter at some, but not all, of the excitatory neuromuscular synapses of the lobster stomatogastric system

The stomatogastric ganglion of the spiny lobster, *Panulirus interruptus*, contains about 30 neurones, 23 of which are motor neurones and make excitatory neuromuscular synapses with 38 pairs of striated muscles which move the lobster's stomach[5] These muscles are typical of crustacea, with fibres approximately 100 μm in diameter and up to several centimetres long According to present knowledge, their sole motor innervation is from the stomatogastric ganglion[5] The motor neurone somata are 40–100 μm in diameter, show both attenuated action potentials and considerable sub-threshold synaptic activity[6], and are identified physiologically by the motor nerve in which their axons are found[7]

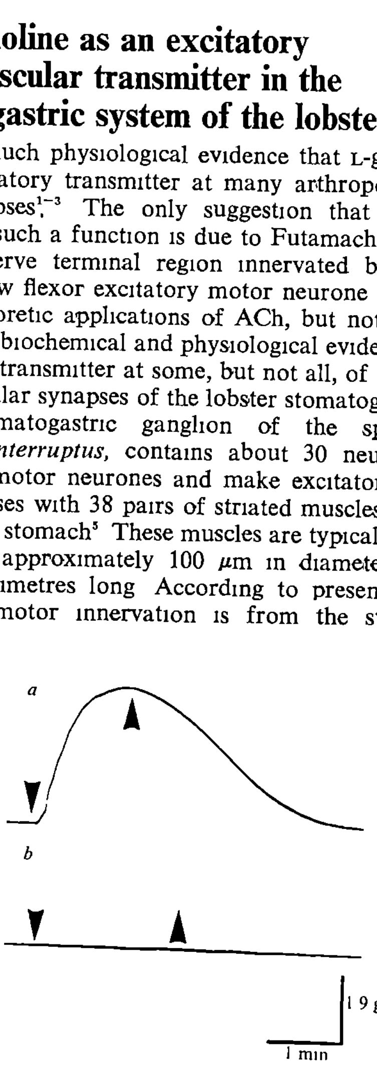

Fig. 1 Effects of bath application of ACh and L-glutamate on a dorsal dilator muscle The muscle was connected to a Grass FT03 force displacement (tension) transducer, and the output was displayed on an oscilloscope and photographed with a kymograph camera The muscle was placed in a 1 5 ml chamber through which saline flowed continuously at 6–8 ml min^{-1} *a*, 2 × 10^{-5}M ACh with 0 01 mg ml^{-1} Tensilon was introduced into the chamber at the downward arrow The muscle contracted, and the upward arrow indicates the start of the wash in normal saline *b*, 1 × 10^{-3}M L-glutamate was introduced to the chamber at the downward arrow, causing no response, and the wash was started at the upward arrow The muscle continued to contract in the presence of ACh for 6 h after the test with L-glutamate

Choline acetyltransferase catalyses the enzymatic biosynthesis of ACh and correlates well with the presence of ACh in *Aplysia* neurones[8] I have assayed this enzyme in individual somata of physiologically identified stomatogastric neurones First, neurone somata were penetrated with microelectrodes for identification of the cells, a drawing was made of their relative positions, and then the cells were dissected individually into small glass tubes by the method of Giller and Schwartz[9], after being frozen in a mixture of 70% ethylene glycol and 30% *Panulirus* saline The tubes containing the cells were kept frozen on dry ice until the assay was performed, usually within 2 d of dissection

Each ganglion contains 2 PD (pyloric dilator) motor neurones and one LP (lateral pyloric) motor neurone[6] Table 1 shows a summary of choline acetyltransferase assays on single PD and LP somata The PD somata contained choline acetyltransferase activity in 16 out of 18 cells assayed The actual values obtained showed a large variation which may reflect damage caused during the identification or dissection, or actual variation in the amount of the enzyme present in different cells The mean of these measurements is 12 6±8 1 pmol ACh per cell per h which may be somewhat low because I made no attempt to discard values from cells which may have been damaged I estimate that the amount of enzyme is about 125 pmol ACh produced per nl cell volume per hour This value is comparable with those found in cholinergic neurones in Aplysia[9]

Table 1 Acetylcholine transferase in single PD and LP somata

	pmol ACh, per cell per h*	No of somata with activity No of somata assayed
PD cells	12 6 ± 8 1	16/18
LP cells	<2 7 ± 0 8	1/10
Limit of sensitivity	2 4 ± 0 7	

* Means plus standard deviations of values from 10 experiments

PD cells showed activity in all but two cells assayed, with a range between 4 0 and 29 9 pmol ACh per cell per hour The LP cells were below the limit of sensitivity in all cases but one, where a low level of activity was found Assays were performed as by Coggeshall *et al*[10] 10 µl of buffer-substrate (final concentrations, 0 1M NaPO$_4$, *p*H 7 9, 0 25M NaCl, 10 mM choline chloride, 1 mM EDTA, 0 1 mM eserine, 16 mM MgCl$_2$ and 0 4 mM^{14}C acetyl CoA, specific activity 5–10 µCi µmol^{-1} (New England Nuclear Corp)) was added to the tubes containing cell somata and to empty tubes (blanks) Tubes were incubated at 38° C for 30 min The reaction was stopped with 100 µl of 50 mg ml^{-1} sodium tetraphenylboron in 3-heptanone Tubes were mixed, 100 µl of 0 2 M NaPO$_4$, *p*H 7 0, was added, mixed and spun in a table-top centrifuge for 2 5 min 50 µl of the organic phase was counted in 10 ml of scintillation fluid Ten to twenty blanks were run with each assay, and the limit of sensitivity was estimated on the basis of the variation in the blanks and the specific radioactivity of the reaction mixture

In all experiments but one, the LP cell showed no acetylcholine transferase activity As the LP cell is frequently the largest cell in the ganglion, and is always one of the largest, this difference in enzyme activity is not a trivial consequence of differences in the size of the PD cells and LP cell within a ganglion

The PD neurones innervate the dorsal dilator muscle (muscle cpv 1a,b according to Maynard and Dando's terminology[5]) Figure 1 shows the effects of bath application of ACh and L-glutamate on the dorsal dilator muscle Figure 1*a* shows the tension produced by 2×10^{-5}M ACh with 0 01 mg ml^{-1} Tensilon (an acetylcholinesterase inhibitor) Dose-response curves show that the threshold for

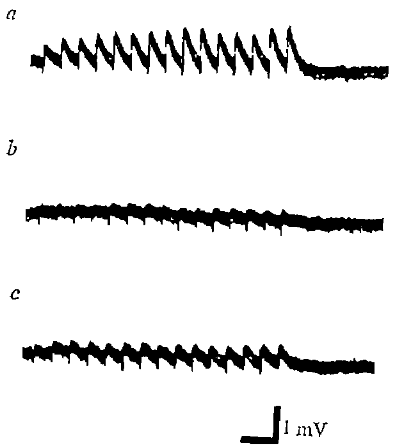

Fig 2 The ejps recorded in a dorsal dilator muscle fibre are reversibly blocked by D-tubocurare The ejps were recorded with a 30 MΩ 2 5M KCl-filled electrode and conventional electrophysiological equipment The ejps were elicited by stimulating the PD motor nerve with a suction electrode in trains of 15 pulses at 10 per s for 1 5 s *a*, ejps recorded in normal saline, *b*, the same penetration 7 min after the addition of 5 × 10^{-5}M D-tubocurare The ejps were almost completely blocked, *c*, the same penetration after 10 min wash with normal saline The ejps returned, although not to the size in (*a*) The resting potential was −64 mV

contraction was 1×10^{6}M ACh and that maximal tension was produced by 2×10^{-5}M ACh D-Tubocurare (1×10^{-4}M) shifted the ACh dose-response curve to the right, characteristic of a competitive inhibitor of the ACh response 5×10^{-7}M α-bungarotoxin (gift from Dr Hannah Friedman) completely blocked the response to 2×10^{-5}M ACh Figure 1*b* shows that 10^{-3}M L-glutamate produced no tension in this muscle, nor did high concentrations (10^{-4}M) of dopamine, serotonin, octopamine, L-aspartate, GABA, glycine, noradrenaline or taurine In a similar experiment the muscle innervated by the LP neurone (p2a)[5] did not contract when 1×10^{-3}M ACh with Tensilon was added, although when the nerve innervating the muscle was stimulated, the muscle did contract[11] These results show that ACh causes contraction in the dorsal dilator muscle while the LP muscle does not respond to ACh

Figure 2 shows that excitatory junctional potentials (ejps) in the dorsal dilator muscle fibres were blocked reversibly by D-tubocurare, a known cholinergic antagonist After 7 min in 5×10^{-5}M D-tubocurare, the normal ejps (Fig 2*a*) were considerably attenuated (Fig 2*b*), after 10 min of washing in normal saline they had recovered, although not completely

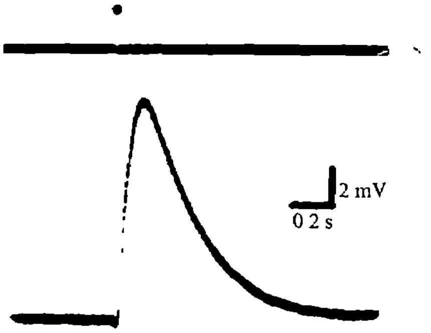

Fig. 3 Iontophoretic application of ACh to the surface of a dorsal dilator muscle fibre The iontophoretic electrode was filled with 3 M ACh and had a resistance of about 90 MΩ A bucking voltage was applied to minimise leakage of the ACh A 30 MΩ electrode filled with 2 5 M KCl recorded membrane potential of the fibre The top trace shows a current monitor The iontophoretic pulse was 100 nA for 20 ms The bottom trace shows the intracellular recording electrode Resting potential was −65 mV Horizontal calibration bar is 200 ms Vertical calibration bar is 100 nA for the top trace and 2 mV for the bottom trace

Figure 3 illustrates the response of a dorsal dilator muscle fibre to iontophoresis of ACh on to its surface Iontophoretic responses of this kind can be recorded from many areas of these fibres The time course and amplitude of the potentials recorded are very sensitive to the position of the ACh electrode, and the ACh response desensitises with repeated applications to the same spot

The data presented here suggest very strongly that ACh is the neurotransmitter at the PD neurone-dorsal dilator muscle synapse, but not at the LP neurone–LP muscle synapse Definitive proof of this requires further experiments, but it is clear now that the PD and LP cells utilise different neurotransmitters Furthermore, it is difficult to argue that L-glutamate is the neurotransmitter at the PD-dorsal dilator synapse, which clearly points to the dangers in making sweeping generalisations about 'the arthropod excitatory neuromuscular transmitter' It is interesting that there is no known peripheral inhibition in the stomatogastric system[12], while many arthropod muscles receiving L-glutamate excitation also receive inhibitory innervation It is also important that the PD and LP neurones make inhibitory synaptic connections within the stomatogastric ganglion[6] There appears to be heterogeneity in excitatory neuromuscular transmitters among the neurones within this one crustacean ganglion, and some of these neurones appear to make cholinergic neuromuscular junctions

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Methylation of ribosomal proteins *in vitro*

STARVATION of an *Escherichia coli rel*$^-$ (RC$^{\mathrm{rel}}$) strain for an essential amino acid results in the synthesis of ribosomal RNA (rRNA) without the concomitant synthesis of proteins[1,2] Ribosomal precursor particles accumulate in these conditions and such precursor particles have been shown to be deficient in the methylation of rRNA (ref 3) We have observed that in addition to proteins L7 and L12, which were previously reported to be methylated[4], several other ribosomal proteins from the 50S subunit were also methylated in the unstarved culture[5,6]. Similar observations have also been made by Alix and Hayes[7], but are the precursor particles generated by *rel*$^-$ strain after starvation for methionine also deficient in the methylation of the ribosomal proteins and if so, can such particles be methylated *in vitro*?

Here we have determined the proteins that were methylated *in vitro*. More than 80% of the incorporated methyl groups was found to be located in one protein (L11). The methylated amino acids present in this protein were also characterised. This information could enable the isolation and purification of protein methylases and the study of the function of protein methylations.

E. coli strain 1500, an *rel*$^-$ mutant of a K-12 strain isolated by Durwald and Hoffmann-Berling[8], was obtained from the Coli Genetic Stock Center in New Haven, Connecticut. The *met B* marker was introduced by P1 transduction from JC-355 (which is a K-12 *met*$^-$ strain originating with A. J. Clark. Cells were grown with a limiting methionine concentration (0.8 μg ml^{-1}) and collected 1.5 h after exhaustion of methionine. During this period of starvation, RNA continues to be synthesised at a linear rate. About 1.2 g of cells were suspended in a buffer containing 0.05 M Tris-HCl, *p*H 7.8, 0.1 mM $MgCl_2$, 0.05 M KCl, and were disrupted with a French press. After centrifugation at 30,000 *g* for 30 min, the supernatant fluid was layered on top of a 5–20% linear sucrose gradient prepared in the same buffer and centrifuged overnight at 18,000 r.p.m. in an SW 25.2 rotor. Fractions containing the 50S peaks were collected and concentrated by ethanol precipitation[9]. Analysis of RNA by the gel electrophoresis procedure of Dahlberg *et al.*[10] revealed that 20% of the total RNA in the pooled 50S particles was in the precursor form. The analysis also showed the presence of less than 4% 16S rRNA which was due to the contamination by 30S subunits. The methylating enzymes were obtained from the 0.5 M KCl wash of 70S ribosomes according to the procedure of Vogel *et al.*[11] from cells grown in the presence of 20 μg ml^{-1} methionine.

Figure 1 shows that the methylation of the 50S ribosomal proteins, as analysed by the one-dimensional polyacrylamide gel electrophoresis, was dependent upon the addition of the methylating enzymes. In the absence of the added enzyme (Fig. 1*a*), very little methylation occurred and the major methylated protein(s) comigrated with a major protein peak at 3.5 cm from the top of the gel. After the addition of the enzymes, methylation of two protein bands at about 2.0 cm from the top was observed (Fig. 1*b*). The latter methylation was dependent upon previous starvation of the culture with methionine as the 50S particle derived from the culture which was not starved with methionine was only methylated to a very small extent in the same conditions.

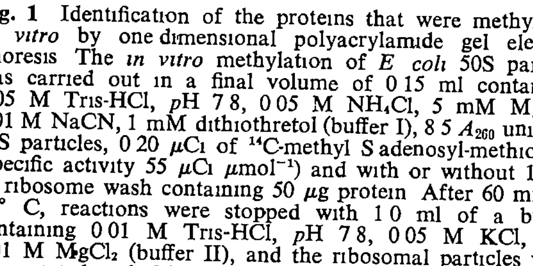

Fig. 1 Identification of the proteins that were methylated *in vitro* by one dimensional polyacrylamide gel electrophoresis. The *in vitro* methylation of *E. coli* 50S particle was carried out in a final volume of 0.15 ml containing 0.05 M Tris-HCl, *p*H 7.8, 0.05 M NH_4Cl, 5 mM $MgCl_2$, 0.01 M NaCN, 1 mM dithiothretol (buffer I), 8.5 A_{260} units of 50S particles, 0.20 μCi of ^{14}C-methyl S-adenosyl-methionine (specific activity 55 μCi μmol^{-1}) and with or without 15 μl of ribosome wash containing 50 μg protein. After 60 min at 37° C, reactions were stopped with 1.0 ml of a buffer containing 0.01 M Tris-HCl, *p*H 7.8, 0.05 M KCl, and 0.01 M $MgCl_2$ (buffer II), and the ribosomal particles were precipitated with 0.75 ml of cold ethanol[9], and dissolved in 40 μl of 4 M urea and 2 M LiCl. After 16 h at 0° C, the ribosomal RNA was removed by centrifugation. The supernatant ribosomal proteins (about 100 μg proteins) were then electrophoresed[13]. After staining of the proteins by amido black, the gels were traced at 650 nm (———) using a linear transporter. The gels were then sliced and autoradiographed[14] and the film was traced at 410 nm (- - -). *a*, The ribosome wash is absent and, *b*, the ribosome wash is present.

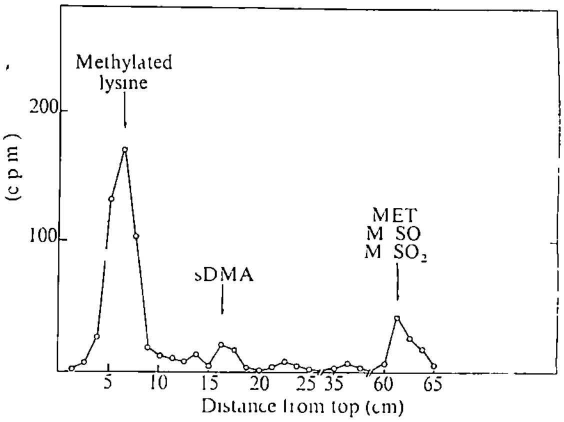

Fig. 2 Analysis of the methylated amino acids derived from protein L11 after *in vitro* methylation of the precursor 50S particle. The reaction conditions were the same as in Fig. 1 except 40 A_{260} units of the 50S particle and 1 μCi of ^{14}C-methyl S-adenosylmethionine in 0.7 ml of buffer I were reacted for 60 min at 37° C. The reaction was stopped with 2.0 ml of buffer II and the ribosomal proteins prepared according to the acetic acid procedure of Hardy *et al.*[15] The ribosomal proteins were lyophilised to dryness and dissolved in 0.15 ml of the sample gel solution used for the first dimension run (*p*H 8.6) of the 2-dimensional electrophoresis procedure of Kaltschmidt and Wittmann[12]. After electrophoresis, protein L11 was cut out from the gel and extracted for 16 h at 30° C with 0.01 M sodium phosphate buffer containing 0.1% sodium dodecyl sulphate (SDS). This was followed by dialysis against three changes of water to remove the excess dye and SDS. The dialysate was filtered to remove the gel and then lyophilised to dryness. The protein was then hydrolysed with 2 ml of redistilled 6 N HCl in a sealed tube at 110° C for 24 h. The hydrolysate was evaporated to dryness under reduced pressure and redissolved in 2 ml of water, followed by evaporation again to dryness. The final pellet was dissolved in 40 μl of water. 20 μl of the above sample were applied to a Whatman No. 3MM (20 × 110 cm) paper together with 10 μg of each of the following standard methylated amino acids: ε-monomethyllysine (MML), ε-dimethyllysine (DML), ε-trimethyllysine (TML), monomethylarginine (MMA), N^G,N'^G-dimethylarginine (uDMA), N^G,N^G-dimethylarginine (sDMA), 1-methylhistidine (1-MH) and 3-methylhistidine (3-MH). The electrophoresis was carried out in a buffer containing pyridine–acetic acid–water (25:1:225), *p*H 6.5, for 3.5 at 4,440 V. After electrophoresis, the paper was sprayed with 0.4% ninhydrin in acetone and the methylated amino acids located by heating for 5 min at 70° C. The oxidised forms of methionine—methionine sulphoxide (M-SO) and methionine sulphone (M-SO_2) migrated with methionine (Met). The paper was then cut into 1.25-cm pieces and counted in a scintillation counter after the addition of 5 ml toluene-based scintillation fluid (4 g Omnifluor per litre toluene).

It was of interest therefore to identify the proteins from the 50S subunit that were methylated *in vitro*. The two-dimensional polyacrylamide gel electrophoresis procedure of Kaltschmidt and Wittmann[12] was used. More than 80% of the incorporated methyl groups was located in protein L11. Proteins L1, L3 and L5 were also labelled to a small extent. Independently, Alix and Hayes[7] have also observed the *in vitro* methylation of protein L11 in cells grown in the presence of ethionine—a methionine analogue.

The nature of the methylated amino acids in protein L11 was also determined. Figure 2 shows protein L11 contains methylated lysine(s) and a very small amount of a compound tentatively identified as N^G,N'^G-dimethylarginine (sDMA). The nature of the methylated lysine was further characterised to be ε-trimethyllysine The presence of ε-trimethyllysine was also verified by the use of the amino acid analyser (C N C , and F N C , unpublished) It should be mentioned that we have also observed the presence of these two methylated basic amino acids in protein L11 obtained from cells grown with ^{14}C-methyl methionine[6] Since the other proteins (L1, L3 and L5) were methylated to a much lesser extent, we have not been able to identify their methylated amino acids

An attempt was also made to calculate the stoichiometry of ε-trimethyllysine in protein following in vitro methylation In the *in vitro* experiment about 55% of the 8,000 c p m incorporated into the 50S particle (8 5 A_{260} units) was found in the protein fraction As 20% of the isolated *rel*$^-$ 50S particles was in the precursor form which was predominately methylated (Fig 1), the amount of incorporation of the methyl groups to protein L11 (which accounts for 80% of the total incorporation into proteins) can be calculated from the specific activity of ^{14}C-cethyl methionine (55 mCi mmol^{-1}) and 50% counting efficiency It was found that there is about 0 30 molecules of ε-trimethyllysine per protein L11 This is lower than the value which we have observed for the *in vivo* methylation of L11 (0 8 molecules/L11) in exponential growth[6] The discrepency between these two results could arise because only a fraction of the precursor particle(s) was methylated or because there was a pool of methylated protein L11 present before the starvation which combined preferentially with the precursor 23S rRNA This would lead to the lower incorporation of methyl groups to protein L11 *in vitro*

We have recently shown that several ribosomal proteins form the 50S subunit (L1, L3, L5, L7, L9, L11, L12, L18 and L33) were methylated *in vitro*[5,6] The failure to observe methylation of some of these proteins (such as L7, L9, L12, L18 and L33) *in vitro* in these experiments could either be due to these proteins being methylated already because they are essential for functioning, or due to the lack of methylases which specifically recognise these amino acids in the above proteins These possibilities are now under investigation Thus, the 50S particle obtained from *rel*$^-$ strain after starvation for methionine should provide a good substrate for the assaying and eventual characterisation of protein methylases

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RNA–RNA hybridisation using ^{125}I-labelled RNA from tobacco necrosis virus and its satellite

SINCE the description by Commerford[1] of a simple method of iodinating nucleic acids, RNA labelled *in vitro* with ^{125}I has been used in structural studies[2], and in DNA–RNA hybridisation studies *in vitro*[3] and *in situ*[4] This technique has considerable potential, especially in studying RNAs which are difficult to label *in vivo*, but most of the information available concerns the labelling of small RNA molecules and there is no information about the stability of large molecules under conditions necessary to obtain high specific activity Furthermore, a recent report[5] suggests that ^{125}I-labelled RNA with high specific activity is degraded on storage and its ability to form DNA–RNA hybrids is lost

Here we show that large plant virus RNA species can be

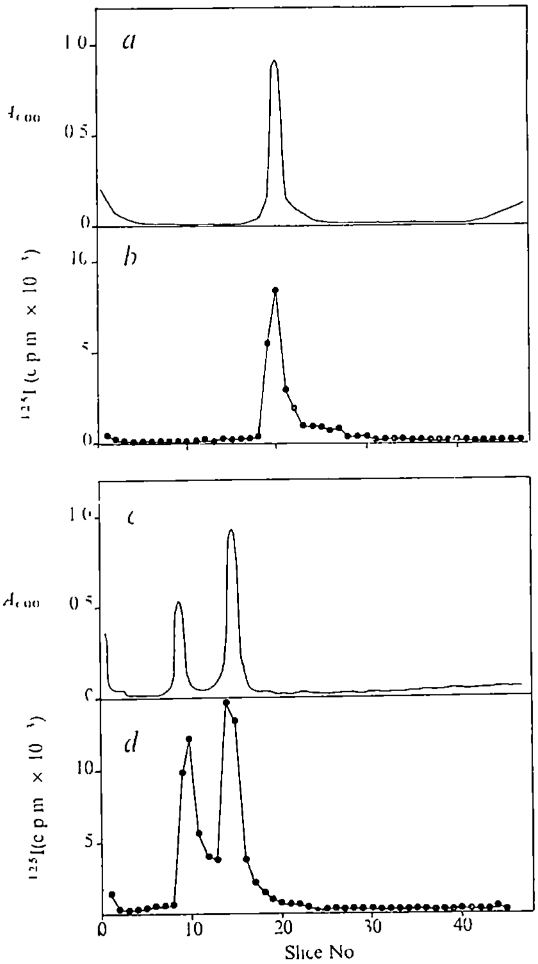

Fig 1 Polyacrylamide gel electrophoresis of RNAs before and after ^{125}I-labelling *a*, SV RNA, *b*, SV RNA labelled for 20 min, *c*, TRV RNA, *d*, TRV RNA labelled for 2 min Labelled and unlabelled RNAs were electrophoresed in parallel for 2 h in 8 cm, 2 4% acrylamide, 0 5% agarose gels containing 0 2% SDS and at 7 5 V cm^{-1} as described by Peacock and Dingman[9] Gels were stained for 1 h in 0 1% toluidene blue in 40% methoxyethanol and destained in 30% methoxyethanol The isotope in the gel slices was determined by scintillation counting

labelled with ^{125}I to high specific activities without significant degradation, that such molecules hybridise fully with complementary strand RNA and that these properties are unaffected by storage We have used this technique to search for nucleotide sequence homology between the RNAs of satellite virus (SV) and its helper, tobacco necrosis virus (TNV)[6]

Conditions for *in vitro* iodination of RNA were adapted from those described by Commerford as follows The reaction mixture in a total volume of 25 μl contained 5 μg RNA, 5 × 10^{-4} μmol KI, 1 2 × 10^{-4} μmol $TlCl_3$, 1 mCi Na ^{125}I (carrier free) and 2 5 μmol sodium acetate, *p*H 4 8 The iodination was at 60° C for 20 min or for shorter periods for very large RNA species which were more susceptible to degradation The reaction was stopped by making the mixture 0 01 M with respect to sodium sulphite, and 0 5 ml 0 05 M Tris, 0 1 M NaCl, 5 mM EDTA was immediately added Yeast RNA (100 μg) was added as carrier and the RNA precipitated by addition of 2 vol ethanol The precipitate was dissolved in 0 25 ml 0 1 M ammonium acetate, *p*H 8 9, and heated at 60° C for 10 min to remove unstable derivatives[1] After addition of 0 25 ml 0 05 M Tris, 0 1 M NaCl, 5 mM EDTA, *p*H 7 2, the RNA was precipitated with alcohol The RNA was further purified by adsorption in 0 01 M potassium phosphate, *p*H 6 8, to a 0 5 ml column of hydroxyapatite, followed by elution with 0 25 M potassium phosphate The overall recovery of RNA was approximately 50%

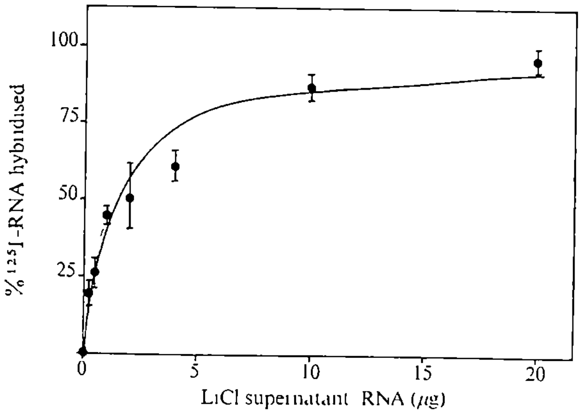

Fig. 2 Hybridisation of ^{125}I-labelled SV RNA with denatured double-stranded SV RNA RNA was extracted from leaves of tobacco plants 3 d after infection with TNV strain A and SV1 (ref 10) as described previously[11] High molecular weight single-stranded RNA was removed by LiCl precipitation[12] and the supernatant used as a source of double-stranded virus RNA[11] This RNA was mixed with 8,000 c p m ^{125}I-labelled SV RNA to a total volume of 0 2 ml in 2 × SSC, and denatured by heating at 115° C for 15 min Annealing was for 20 h at 70° C TCA-precipitable ^{125}I was measured after ribonuclease digestion[11] Each point is the mean of three determinations The solid line drawn is theoretical, and describes the relationship between the concentration of denatured double-stranded RNA and the fraction of single-stranded RNA hybridised under conditions where the reaction is complete[13]

Using this method iodination for 20 min yielded RNA with a specific activity in the range 3 × 10^7 to 8 × 10^7 d p m μg^{-1} Iodination for 2 min gave RNA with a specific activity between 5 × 10^6 and 3 × 10^7 d p m μg^{-1} Figure 1 shows the electrophoretic behaviour of these RNAs on polyacrylamide gels before and after iodination SV RNA (molecular weight 0 4 × 10^6)[7] is undergraded after iodination for 20 min The two RNAs of tobacco rattle virus (molecular weights 2 5 × 10^6, 0 7 × 10^6)(ref 8) were essentially intact after iodination for 2 min, but the larger of the two RNAs was considerably degraded during a 20 min labelling period The behaviour of the iodinated RNA on acrylamide gel electrophoresis was unaltered after storage for 3 months as an ethanol precipitate at −20° C

The ability of these labelled RNAs to hybridise with complementary strand RNA was examined by annealing them with denatured double-stranded virus RNA extracted from virus-infected plants Figure 2 shows the results of annealing ^{125}I-RNA of SV strain 1 with increasing concentrations of denatured double-stranded SV1 RNA At high concentrations of double-stranded RNA nearly all the ^{125}I is ribonuclease resistant The data fit well to the line drawn, which is theoretical and describes the relationship between the concentration of denatured double-stranded RNA and the fraction of single-stranded RNA hybridised under conditions where the reaction is complete[13] The results shown in Fig 2 were obtained with ^{125}I-RNA which had been stored for 3 months

These techniques have been used to look for common nucleotide sequences in TNV RNA and SV RNA The total dependence of satellite virus on TNV for replication[6] is thought to be due to a requirement by SV RNA for the RNA-dependent RNA polymerase specified by TNV and it has been suggested that TNV RNA and SV RNA may contain common nucleotide sequences which act as recognition sites for such a shared polymerase[14] The two RNAs are known to contain an identical trinucleotide at their 5′ termini[14-16], and Klein and Reichmann[17] reported that at least 10% of the SV RNA genome was homologous with that of TNV

In an experiment in which ^{125}I-labelled SV RNA was hybridised to denatured double-stranded SV RNA, in the absence of any competitor RNA, approximately 3,000 c p m of ^{125}I-labelled RNA was hybridised When unlabelled SV RNA (10^{-13} mol) was included as a competitor, hybridisation was reduced by about 90%, whereas the same molar concentration of TNV RNA had no detectable effect, indicating a lack of sequence homology between these RNAs A more sensitive approach is to attempt to anneal ^{125}I-labelled SV RNA with denatured double-stranded TNV RNA (Table 1) Less than 0 5% of the ^{125}I-labelled SV RNA hybridised to TNV complementary strand RNA in this experiment Since this concentration of TNV complementary strand was sufficient to anneal with 23% of the ^{125}I-labelled TNV RNA it follows that less than 2% of the SV RNA sequences are homologous with TNV RNA sequences Any sequence homology which may exist between these two RNAs is therefore limited to a total of less than 30 nucleotides, and this is difficult to reconcile with an earlier report of considerable sequence homology between these RNAs[17]

Table 1 Attempts to form hybrids between SV RNA and TNV RNA complementary strand

Source of double-stranded RNA	^{125}I-labelled RNA (c p m)	^{125}I-hybridised (c p m)
Uninfected plants	TNV (8,650)	151 ± 23 (3)
TNV infected plants	TNV (8,650)	2070 ± 120 (3)
Uninfected plants	SV (2,330)	43 ± 2 7 (6)
TNV infected plants	SV (2,330)	38 ± 1 6 (6)

Denatured LiCl supernatant RNA (100 μg) from either uninfected or TNV infected plants was annealed with ^{125}I-labelled SV or TNV RNAs, the iodinated RNAs being used at the same molar concentrations Experimental methods were as described in Fig 1 The mean values for the quantities of ^{125}I hybridised are given with their standard errors, and the figures in brackets in this column refer to the number of replicates in each case No detectable ^{125}I-labelled SV RNA was annealed by TNV complementary strand RNA (< 0 5% $P > 0 99$)

We have shown here that iodinated RNA has considerable potential in RNA–RNA hybridisation studies It should be particularly useful to plant virologists since pure virus RNAs are often readily available, but isotopically labelled RNA is particularly difficult to obtain by *in vivo* labelling methods

We thank Vicky Maxwell for technical assistance and Mr David Smith for assistance in propagating TNV We thank Drs D Barbara and B Kassanis for helpful discussion Purified satellite virus was a gift from Dr B Kassanis This work was supported by the Agricultural Research Council

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Double helix formed by the dinucleoside phosphate thymidylyl-3′,5′-deoxyadenosine

IN our conformational studies of nucleic acid components we have obtained X-ray diffraction photographs from specimens of the dinucleoside phosphate thymidylyl-3′,5′-deoxyadenosine (TpdA) which are similar to those obtained from certain synthetic DNA copolymers The dinucleoside phosphate was obtained from International Enzymes Ltd as the triethylammonium salt Some of it was dissolved in a small quantity of water between a glass slide and a coverslip resting on two thin glass fibres Some of the material which dried out under the coverslip showed evidence of orientation when viewed between crossed polaroids X-ray diffraction photographs were taken on a microcamera using a Hilger and Watts semi-microfocus X-ray generator and one of these is shown in Fig 1

The diffraction patterns are similar to those obtained from fibre specimens of nucleic acids and they are characteristic of helical diffraction patterns, with an integral number of residues per turn of the helix The layer-line spacing at ~57% relative humidity is 26 3 Å There are discrete reflections only on the equator and these can be indexed on a hexagonal lattice with a=21 25 Å Both layer-line spacing and the equatorial pattern depend on water content and at ~92% relative humidity the layer-line spacing is 27 2 Å and a=22 7 Å The pattern is characterised by a very strong intensity on the seventh layer line close to the meridian, by a strong first layer line and a weaker second layer line These characteristics are similar to those observed in some of the fibre X-ray diffraction patterns of the synthetic double-helical copolymers poly d(A-T) poly d(A-T) (ref 1) and poly d(I-C) poly d(I-C) (ref 2)

The similarity in the diffraction patterns obtained with TpdA and the polymers can be explained if two TpdA molecules are related to each other by a twofold rotation axis so that they are linked to each other by Watson–Crick pairing of bases in a similar way to that observed in the single-crystal structure of the dinucleoside phosphates adenylyl-3′,5′-uridine (ref 3) and guanylyl-3′,5′-cytidine (ref 4) Pairs of TpdA molecules can then stack on top of each other to produce an extended helical structure The structure would thus be similar to a double helix in which alternate phosphate groups would be missing The fact that the backbone of such a helix is discontinuous suggests that stacking forces are important in stabilising the structure and in determining its integrity

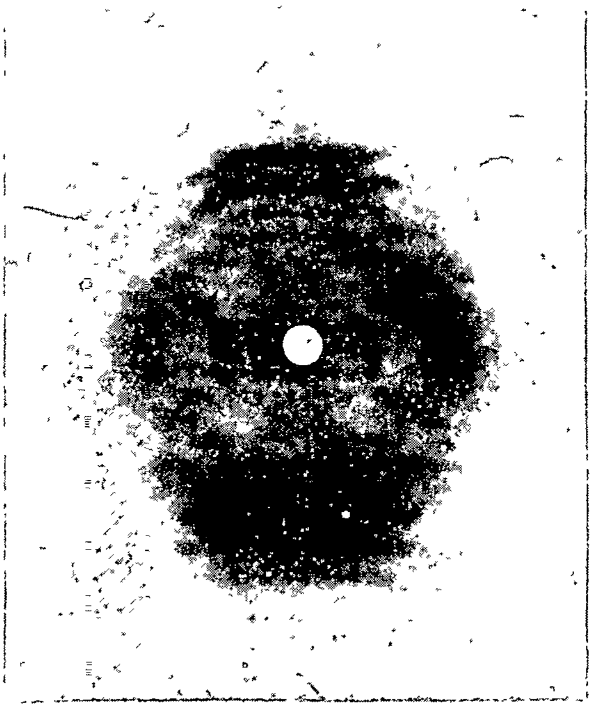

Fig 1 X-ray diffraction photograph of TpdA at 57% relative humidity

The intensity distribution on the fifth, sixth, seventh and eighth layer lines is similar in some ways to the distribution in the corresponding region of the diffraction pattern of the A-form of DNA[5], suggesting that the bases are tilted from perpendicular to the helix axis The tilt is 20° in A-DNA, where the rise per nucleotide along the helix is 2 56Å In TpdA, an estimate of the number of molecules in the 26 3 Å repeat gives a value of 12 or 10,—an odd number of molecules would mean a doubling of the repeat period which is not observed Twelve molecules would correspond to a stack of six pairs of molecules with a rise of 2 19 Å from one base to the next along the helix Ten molecules correspond to a stack of five pairs of molecules and a rise of 2 63Å, which seems a more reasonable value when compared with polynucleotide structures Further analysis of the diffraction patterns should clarify this point and should enable a detailed structure determination to be made It will be of interest to compare the TpdA structure with that recently proposed for poly d(A-T) poly d(A-T) (ref 6)

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Specific binding of antitumour drug *cis*-Pt$(NH_3)_2Cl_2$ to DNA rich in guanine and cytosine

ONE of the more effective *cis*-platinum antitumour compounds currently being tested in cancer chemotherapy is *cis*-Pt$(NH_3)_2$ Cl_2 (ref 1) Evidence suggests that DNA is the effective site of action of the platinum drug[2,3] This study was initiated to determine the effective binding site on DNA Roberts and Pascoe have noted the similarity of action between *cis*-Pt $(NH_3)_2Cl_2$ and the classical antitumour bifunctional alkylating agents which crosslink DNA (ref 2) Low concentrations of several *cis*-platinum compounds suppress DNA synthesis *in vitro* while affecting protein and RNA synthesis to a much lesser extent[3] The studies by Horacek and Drobnik employing ultraviolet absorption spectroscopy indicate that the platinum moiety binds to the bases of DNA rather than to the phosphate backbone[4] Spectroscopic studies suggest that *cis*-Pt$(NH_3)_2$ Cl_2 preferentially binds to guanine–cytosine (G–C)-rich DNA[5,6] An increase in the buoyant density of T7 DNA and calf thymus DNA has been reported when one of several platinum compounds is present in solution[5,7] Here we report that the binding of *cis*-Pt$(NH_3)_2Cl_2$ to DNA results in large increases in buoyant density which are proportional to the G–C content of the DNA and to the molar ratio of platinum to DNA-P

Rat liver DNA (42% G–C), radiolabelled with ^{3}H-thymidine, and *M lysodeikticus* DNA (72% G–C), both prepared by standard methods[8], were mixed in 0 015 M sodium chloride Concentrations of the original DNA solutions were determined using the molar extinction coefficients at 260 nm of 6650 for rat liver DNA and 6700 for *M lysodeikticus* DNA (ref 9) Solutions containing 10^{-3} M *cis*-Pt$(NH_3)_2Cl_2$ in 0 015 M sodium chloride were added and the mixture incubated at room temperature in the dark Buffers were avoided because of their potential metal-binding properties[10] Periodic measurements during the course of the reaction indicated a *p*H of about 6 5 The ultraviolet absorption parameter $A_{250\,nm}/A_{270\,nm}$, an indication of platinum binding to DNA (ref 4), was used to monitor the reaction This ratio decreases until equilibrium is established In the conditions employed, this required about 1 d

Aliquots containing 10–30 μg of platinised DNA were then mixed with caesium sulphate solution and banded in an isopycnic gradient The refractive index and absorbance at 260 nm of sequential 0 3 ml fractions were determined and densities were derived using the formula of Vinograd[11] The radioactivity of each fraction was determined using Aquasol (New England Nuclear) and a Hewlett Packard liquid scintillation counter Some gradient fractions were dialysed to remove the caesium salt and the ultraviolet absorption spectrum of the recovered DNA was determined The ratio $A_{250\,nm}/A_{270\,nm}$ indicated that the molar ratio of DNA-bound platinum to DNA-P had remained unchanged during the isopycnic banding procedure

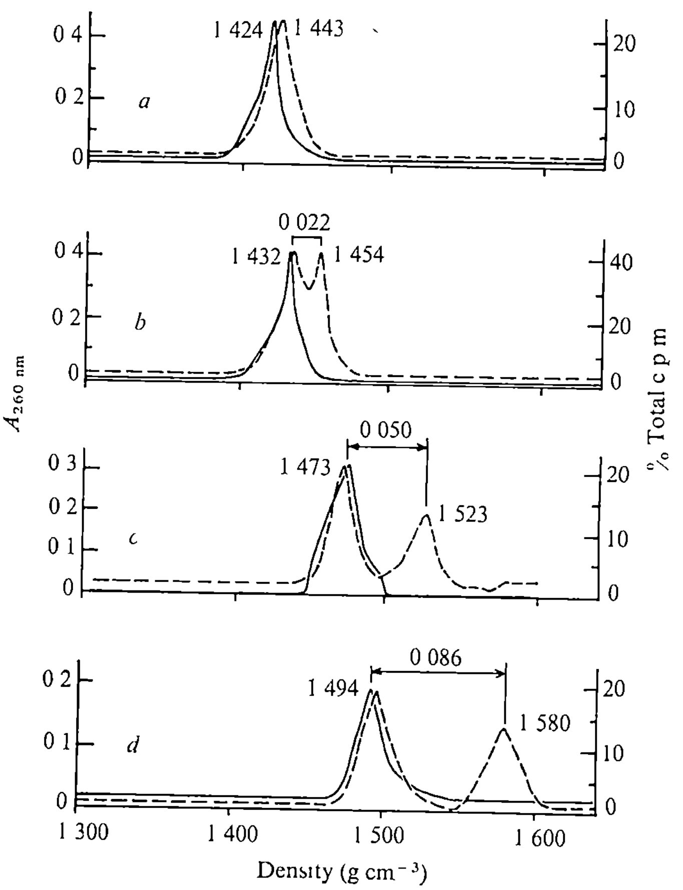

Fig 1 Rat liver DNA, radiolabelled with ^{3}H-thymidine, was mixed with *M lysodeikticus* DNA, reacted with various amounts of *cis*-Pt$(NH_3)_2Cl_2$ and analysed in a caesium sulphate isopycnic density gradient The gradients were fractionated and the refractive index, the absorbance at 260 nm (----) and the radioactivity (——) were determined as described in the text The buoyant densities of unreacted rat liver DNA and *M lysodeikticus* DNA were determined individually as 1 424 g cm^{-3} and 1 443 g cm^{-3} respectively The concentrations of rat liver DNA, *M lysodeikticus* DNA and *cis*-Pt$(NH_3)_2Cl_2$ used were *a*, 9 20 × 10^{-5} M (DNA-P), 1 17 × 10^{-4} M (DNA-P), 0, *b*, 8 40 × 10^{-5} M, 1 06 × 10^{-1} M, 9 90 × 10^{-6} M, *c*, 7 70 × 10^{-5} M, 9 72 × 10^{-5} M, 1 67 × 10^{-5} M, *d*, 6 93 × 10^{-5} M, 8 74 × 10^{-5} M, 2 57 × 10^{-5} M

Figure 1 illustrates the increasing separation of rat liver DNA and *M lysodeikticus* DNA at higher concentrations of *cis*-Pt$(NH_3)_2Cl_2$ *M lysodeikticus* DNA increases in density more than ^{3}H-thymidine-labelled rat liver DNA, which has a lower G–C content To rule out the possibility that *M lysodeikticus* DNA remained in the low density band, the experiment was repeated without rat liver DNA We obtained a single homogeneous peak for *M lysodeikticus* DNA and an increase in density similar to that obtained in the previous experiment When the experiment is performed without *M lysodeikticus* DNA a single homogeneous peak is also obtained for rat liver DNA

The effects of *cis*-Pt$(NH_3)_2Cl_2$ on other DNAs were determined in a similar manner The findings are presented in Fig 2 The buoyant density increase of each DNA is proportional to its G–C content and also depends on the molar ratio of platinum to DNA-P Similar results have been obtained using caesium chloride gradients

We wished to determine how firmly the platinum compound was bound to the DNA Exhaustive dialysis of platinised DNA against some of the usual chelating agents such as EDTA or penicillamine or in solutions containing high concentrations of such anions as sulphate or chloride failed to reverse the buoyant density and spectroscopic changes induced by treatment of DNA with the platinum compound Preliminary

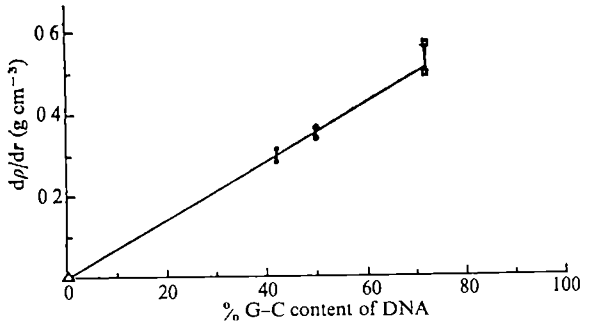

Fig. 2 The relationship between the increase in density of a DNA, treated with *cis*-Pt$(NH_3)_2Cl_2$, and the base composition of that DNA The increase in buoyant density, *d*ρ, of various DNAs as a function of the increase in the molar ratio of platinum to DNA-P, *dr*, is presented as *d*ρ/*dr* versus G–C content of the DNA *Cis*-Pt$(NH_3)_2Cl_2$ was reacted with individual DNAs in 0 015 M sodium chloride to completion The buoyant density was determined as described in the text and the value of *d*ρ/*dr*, the platinum dependent density change for the DNA, was obtained from a plot of buoyant density versus molar ratio of platinum to DNA-P To determine whether or not *d*ρ/*dr* was altered by the presence of another DNA, a second procedure was used which yielded similar values Equal amounts of a test DNA and a reference DNA, for which *d*ρ/*dr* was known, were reacted with *cis*-Pt$(NH_3)_2Cl_2$ in a 0 015 M sodium chloride solution These DNAs were banded in caesium sulphate as described in the text Then, $(d\rho/dr)$ test DNA $=(d\rho/dr)$ reference DNA $\times \dfrac{\Delta\rho \text{ test DNA}}{\Delta\rho \text{ reference DNA}}$ For example, from Fig 1, the increase in buoyant density for *M lysodeikticus* DNA is 1 8 times the increase in density for rat liver DNA from the same solution Therefore $(dp/dr)_{M\ lysodeikticus\ \mathrm{DNA}} = 0\ 30\mathrm{g\ cm}^{-3} \times 1\ 8 = 0\ 54\,\mathrm{g\,cm}^{-3}$ The value of 0 30 g cm^{-3} for rat liver DNA, in this case, was determined by the increments in density that occurred when this DNA alone was reacted with various concentrations of the platinum compound Values of *d*ρ/*dr* were obtained for (poly (dA) (poly (dT)) and poly (dA-T)[2] (Δ) calf thymus DNA (42% G–C) and rat liver DNA (42%G–C) (●) *E coli* DNA (50% G–C) (○), and *M lysodeikticus* DNA (72% G–C, (□)

indications, however, are that most, but not all, of the DNA-bound platinum can be removed by dialysis for several weeks with 10^{-3} M solutions of potassium cyanide and 0 015 M sodium chloride It is not presently known whether or not removal of the DNA-bound platinum results in nicking or other damage to the DNA

Understanding of the action of *cis*-Pt$(NH_3)_2Cl_2$ may result in the development of more useful analogues of these antitumour agents In addition, isolation of guanine rich regions of fragmented DNA might be facilitated using the larger increase in density which occurs with platinisation

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RNase inhibition of reverse transcriptase activity in human milk

The aetiological role of RNA tumour viruses in mammary cancer of experimental animals coupled with observations of morphologically similar particles in human milk[1,2] has motivated considerable interest in the biological role of these virions in man The specificity and sensitivity of the simultaneous detection test[3] for reverse transcriptase (RT) which requires that the viral enzyme polymerise radioactive DNA using endogenous 70S and/or 35S viral DNA as template, suggested its usefulness for the detection of RNA tumour viruses in human milk[4] In two recent studies[2,5] comparing the detection of such virions in human milk by electron microscopy (negative staining) with the detection of RT activity, no correlation was observed As a part of our effort at relating the presence of such virions in human milk to a familial history of breast cancer, we employed reconstruction experiments to determine the reliability of the simultaneous detection test for the routine testing of human milk samples for the presence of RNA tumour viruses. Analysis of the products of the reaction was carried out by the use of gel electrophoresis instead of by velocity gradients[6] This method offers the advantage that, in addition to the 70S and/or 35S viral peak, it also detects a 4S peak which represents the DNA products of the reaction which are free from the large molecular weight viral RNA

Using this modification of the simultaneous detection test, we have repeatedly and reproducibly detected the presence of RNA tumour viruses from a variety of sources including some samples of human milk Experiments describing the high degree of proportionality and reproducibility readily attained with non-milk sources will be described elsewhere

In our reconstruction experiments, 3×10^4 focus forming units (FFU) of murine leukaemia virus (MuLV) (Rauscher) supplied by University Laboratories and assayed by them in FFU of helper virus) was added to each of 25 human milk samples obtained from healthy donors Under these conditions, any endogenous RT activity would be expected to contribute less than 10% of the total counts and thus not be a source of confusion Figure 1 shows the electrophoretic profiles of the products of the simultaneous detection test Profile *a* gives the products of the MuLV control diluted in 0 85% NaCl Four of the 25 samples gave profiles identical to this profile Profile *b* was seen in 17 of the 25 samples There is no 35S peak and little or no 4S peak Profiles *c* and *d* with reduced 35S and 4S peaks were seen in 4 out of 25 samples Similar results were obtained when avian myeloblastosis virus (AMV) was substituted for MuLV in this series of reactions It is clear, therefore, that the majority of the milk samples tested inhibited the detection of the reverse transcriptase reaction of at least two known RNA tumour viruses

We had previously observed profiles similar to these in Fig 1*b*, *c* and *d*, when, before beginning the enzymatic reaction, we had deliberately added RNase to the viral pellet to demonstrate the RNA dependency of the reaction Therefore, we made a direct examination for the presence of RNase in the viral pellet prepared from the milk samples described above Table 1 shows the relationship between RNase activity in the viral pellet from milk and the inhibition of RT activity of the same pellet The positive correlation between the presence of RNase in the viral pellet and the inhibition of the RT activity supports the suggestion that RNase in human milk is a significant inhibitor of the simultaneous detection test for RT It was not possible to examine these particular milk samples further since the limited amount of milk available was used in these assays

A similar interference with the RT activity of MMTV in milk from RIII mice by an inhibitor present in human milk was reported earlier[4] These workers also reported that on some occasions trypsin pretreatment could be shown to increase the RT activity detectable in human milk samples We tested trypsin pretreatment of human milk containing added MuLV and found that it rarely prevented inhibition of RT Similarly we found that it failed to lower the measured level of RNase present in such milk samples (J J McC, L J L, and M A R, unpublished)

Since the simultaneous detection test was inadequate for screening milk samples, we investigated whether another RT assay might offer a valid approach The use of exogenous synthetic templates, such as poly (A) oligo (dT) and poly (dA) oligo (dT) or poly (C) oligo (dG) (ref 7), to assay RT activity provided an extremely sensitive assay, but this reaction was also inhibited when reconstruction experiments with MuLV or AMV added to milk were carried out and the test run on the viral pellet

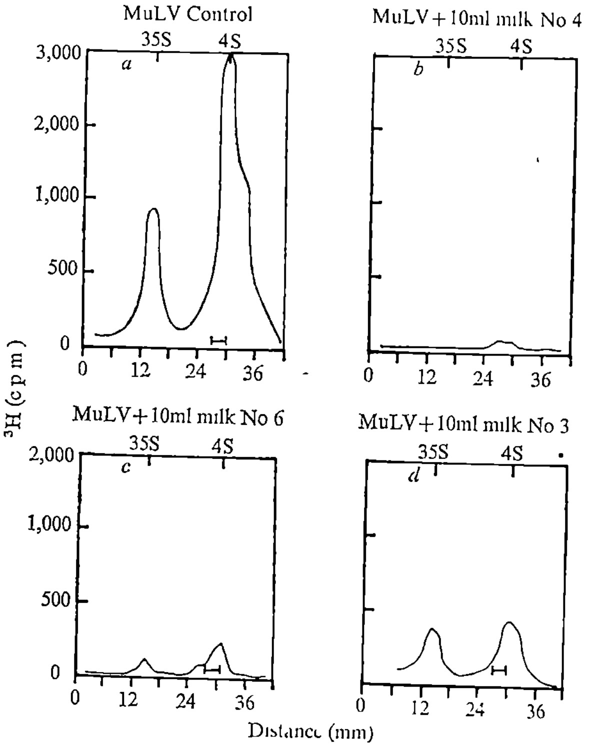

Fig 1 Twenty five human milk samples were 'seeded' with 3×10^4 FFU of MuLV assayed for RT activity according to the procedure of Schlom *et al* [4], (with the minor modifications described below) and the products analysed by polyacrylamide gel electrophoresis[6] The peak between fraction 12 and 14 represents the 35S viral RNA which is acting as template in the reaction The peak between fractions 24 and 36 represent the DNA products of the reaction which are no longer bound to the 35S RNA Four of the 25 samples gave profiles identical to that in *a* (MuLV assayed independently of milk), 17 of the 25 gave profiles like *b*, and 4 gave profiles like *c* and *d* 15 ml of milk containing added MuLV was diluted with 7 ml of 10 mM Tris-HCl, 150 mM NaCl, 2 mM mgCl, (*p*H 7 4) (TNM) and centrifuged at 4,000*g* for 10 min The clear 'milk-plasma' zone between the lipid and the pellet was removed and overlaid on a 8 ml glycerol pad and centrifuged at 4° C for 1 h at 98,000*g* in a Spinco SW 27 rotor to form the 'viral pellet' Centrifugation of the milk-TNM mixture at 4,000*g* for 10 min and of this supernatant at 16,300*g* for 10 min to clear the large molecular weight material before the centrifugation at 98,000*g* was not effective in enhancing the reverse-transcriptase activity of the viral pellet or of lowering the RNase activity of this pellet The use of EDTA instead of TNM did not aid in the precipitation of casein in human milk and inhibited the RT reaction of MuLV or AMV as has been noted[12] The RT assay was carried out with an incubation at 37° C for 1 h (identical curves showing about 25% as much incorporation were obtained with 15 min incubations)

Table 1 RNase and reverse transcriptase in human milk seeded with MuLV

Milk no	RNase	RT activity (% of control)
1	+	0
2	+	20
3	+	43
4	+	0
5	+	0
6	+	13
7	+	24
8	−	87
9	+	0
10	+	0
11	+	0
12	−	100
13	−	100
14	−	100
15	+	0
16	+	0
17	+	0
18	+	0
19	+	0
20	+	0
21	+	0
22	+	0
23	+	0
24	+	0
25	+	0

Reverse transcriptase activity (% control) was determined as in Fig 1 The control was 3×10^4 FFU of MuLV seeded into 15 ml of 0 85% NaCl To determine the presence of RNase activity, 0 005 ml of the viral pellet immediately after resuspension in 0 050 ml of 10 mM Tris-HCl (*p*H 8 3) was added to tritiated mouse ribosomal RNA and incubated at 37° C for 1 h Glycerol was added to a concentration of 10% and the sample subjected to electrophoresis on a 1 8% acrylamide 0 5% agarose gel for 60 min at 2 mA per gel Gel sections were cut, hydrolysed in 30% peroxide at 60° C and counted in Aquasol in a Packard liquid scintillation spectrometer A sample was designated RNase negative if a sharp 28S RNA peak containing 75% or more of counts of the control was found The 28S peak was absent from all samples designated RNase positive

The other type of reverse transcriptase assay in common use is the endogenous template method[8] This is a less refined version of the simultaneous test in which all of the radioactive products of the reaction are precipitated together Such a method would also be unsatisfactory for the assay of MuLV or AMV seeded human milk samples since, as shown in Fig 1 in 84% of the samples tested the total amount of radioactive product is significantly lower than the control value

If RNase present in human milk is, indeed, inhibiting the reverse transcriptase activity of virus added to such a sample, it should be possible to demonstrate this by determining the amount of RNase present and then showing that adding this amount of RNase to a reverse transcriptase reaction in buffer causes the same amount of inhibition as adding the milk sample itself To determine the amount of RNase present in the reverse transcriptase reactions of a number of milk samples, we centrifuged them, as described for Fig 1, using the procedures we employed for preparing 'viral pellets'. We resuspended such pellets in 0.01 M Tris-HCl *p*H 8.3 in 1/100 the original volume and determined the amount of RNase present in aliquots of this solution using ribosomal RNA as described in Fig 2. We then determined how much inhibition of a virus RT reaction in buffer, using the template poly (C) oligo (dG), was caused by a particular volume of this resuspended milk pellet. To equate this inhibition with the concentration of RNase present, equivalent concentrations of pancreatic RNase were added to a parallel series of viral RT reactions and the percentage inhibition determined. Since mouse mammary tumour virus (MMTV) may be a better model for a human mammary virus than leukaemia virus, these assays were done with MMTV purified from RIII mouse milk[8] as well as with MuLV. The results are shown in Fig 2.

Milk samples 33 and 39 show the same degree of inhibition of the RT reaction with MMTV or MuLV as was obtained when the equivalent amount of pancreatic RNase was added to the MMTV RT reaction. Low levels of pancreatic RNase added to MuLV show a similar RT inhibition, but the RT appears to be more resistant to higher RNase concentrations. The viral pellet of sample 33 contains 2 ng RNase ml^{-1} of milk used to prepare the pellet and sample 39 contains 10 ng RNase ml^{-1} of milk used. From these data it is clear that the inhibition of RT activity by human milk samples can be accounted for by the RNase activity of the viral pellets.

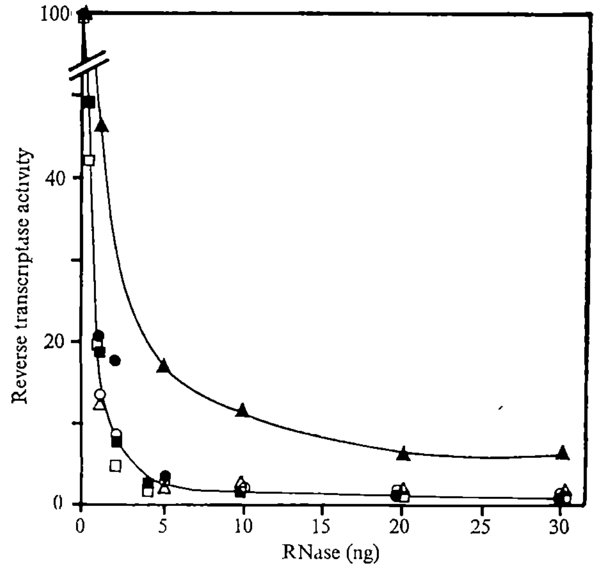

Fig 2 The inhibition of a synthetic template reverse transcriptase reaction by the addition of pancreatic RNase or milk 'viral pellet' which contains the same amount of RNase. The human milk was prepared as described in the legend of Fig 1, and the 'viral pellet' resuspended in 10 mM Tris-HCl (*p*H 8.3) in 1/100 the original volume. RNase was assayed quantitatively by measuring the TCA-precipitable counts after incubating tritiated mouse ribosomal RNA with the aliquots of the resuspended 'viral pellet'. Incubation was for 10 min at 0° C. 10% TCA was added to stop the reaction and 50 μg of yeast RNA was added as carrier. Samples were precipitated on cellulose nitrate filters, dried, and counted in a toluene–PPO–POPOP cocktail in a Packard liquid scintillation spectrometer. The units of RNase per test sample were determined by comparison with the TCA precipitable counts remaining after exposure of the RNA to a known amount of commercial bovine pancreatic RNase A (80 Kunitz units mg^{-1}) (Worthington Biochemical Corporation, Freehold, New Jersey). Aliquots of ▲, pancreatic RNase (dissolved in 10 mM Tris-HCl, *p*H 8.3), ●, the resuspended 'viral pellet' of human milk sample 2 33 (2 ng of RNase ml^{-1} of milk used) ■, human milk no 39 (10 ng of RNase ml^{-1} of milk used), each containing equal amounts of RNase activity, were added to RT assays containing 3 × 10^{3} FFU of MuLV and 5 μg ml^{-1} of the synthetic template (poly rC) (oligo dG) and the reaction carried out as described. An identical series of reactions was also carried out with MMTV in the RT assay (△, ○, □). The fact that a similar inhibition of the reaction is seen when identical levels of RNase are added demonstrates that the RNase present in the milk 'viral pellet' is sufficient to explain the inhibition of such reactions seen when MuLV, AMV or MMTV are added to human milk in reconstruction experiments.

It is not immediately clear how RNase, a small molecular weight protein, can reach the viral pellet since the centrifugation conditions (98,000*g* for 1 h over a 8 ml pad of 20 or 30% glycerol) are clearly not sufficient to pellet RNase. We have noted, however, that the milk samples centrifuged in these conditions form a large pellet. Since casein, a common milk protein, can form micelles of sufficient mass to pellet in these conditions, we examined the viral pellet for casein by the Ouchterlony double diffusion technique against rabbit anti-human casein. Human casein was readily detected in these pellets suggesting that RNase could indeed reach the viral pellet as part of a casein micelle.

We have found that the amount of RNase in the resuspended 'viral pellet' is proportional to the volume of human milk used and that the variation of RNase present in a group of milk samples tested was between 2 and 20 ng ml^{-1} (1 mg of RNase is equal to 80 Kunitz units (ref 9)). In the synthetic template RT assay shown in Fig 2, 3.0×10^{3} FFU of MuLV virus, which we have shown by laser light scattering contains about 0.5×10^{7} viral particles[10], is 95% inhibited by 2.5 ng of RNase. Assuming that the RT activity of the virus found in human milk using this template is as sensitive to RNase as MuLV or MMTV, a human milk sample would have to contain more than 0.5×10^{7} endogenous viral particles ml^{-1} if it contained 2 ng of RNase ml^{-1} and more than 1×10^{8} particles if it contained 20 ng of RNase ml^{-1} to exhibit positive RT activity with a synthetic template test. We have verified these figures by the addition of suitable amounts of MuLV to human milk and found that when virus is in excess, positive RT's are obtained as predicted.

The milk of various strains, especially RIII, is well known to contain large amounts of MMTV and such samples have routinely given positive RT assays. We have found that the RNase activity of the 'viral pellets' of RIII mouse milk falls in the same range as that from human milk samples, but that the viral content of such samples as determined by laser light scattering is 25–500 times higher than the amount of virus added to human milk in our reconstruction experiments. It is clear that the large difference in the number of viral particles can account for the high frequency of positive RT assays with RIII mouse milk as opposed to human milk.

The data presented here may well account for the lack of correlation between RT activity in human milk, and the detection of virus particles by electron microscopy, or the epidemiological risk to breast cancer[2,5,11]. Attempts to assess the RNase sensitivity of endogenous RT activity in human milk and to develop an inhibitor or RNase compatible with the detection of RT activity are in progress.

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Capacity of complement C3 phenotypes to bind on to mononuclear cells in man

SINCE the discovery of the genetic polymorphism of C3, the third component of human serum complement[1-3], no demonstration of functional differences between the common phenotypes F and S has been published Total or specific C3 haemolytic activity has been found to be the same for C3 variants[1,4] as was also the case with immune adherence titres in sera with different C3 phenotypes[1]

Evidence has accumulated about the complement receptors in the membrane of leukocytes in various animal species including man[5,6] The complement component involved in the binding to the leukocytes seems to be C3 (ref 5) It seemed worthwhile investigating the possibility of genetically determined differences in C3 molecules showing in this potentially significant immunological function of C3 Here evidence is put forward that there are functional differences in man between the common phenotypes F and S in the capacity to bind with the complement receptor on mononuclear cells *in vitro*

Mononuclear cells from normal human individuals were obtained by Ficoll-Isopaque centrifugation of defibrinated peripheral blood and washed twice by centrifugation at 200*g* with Hanks's basal salt solution (HBSS) and resuspended in HBSS C3 phenotypes of healthy laboratory staff and blood donors were determined by starch gel electrophoresis as described previously[7] Complement was coupled on to erythrocytes by two methods First, the method of Jondal *et al* [8] was used Human blood group O red cells were coated with antibody by incubating with inactivated rabbit anti human O red cell serum diluted 1 1000 For the binding of complement, human sera at a dilution of 1 10 were incubated with the erythrocyte-antibody complex (HEAC-technique) Secondly, low ionic strength was used in order to coat red cells with complement[9] One millilitre of 5% human erythrocyte suspension in saline was spun down and the supernatant discarded One drop of fresh human serum was added to the packed cells followed by 1 ml 10% sucrose (w/v) The mixture was incubated at 37° C for 15 min Then the cells were washed three times with saline and finally suspended in 2 ml of HBSS (HEC-technique)

Table 1 The effect of C3 phenotype on the number of HEC rosette-forming mononuclear cells and the concentration of serum C3

	C3 phenotype S	C3 phenotype F
No of sera studied	9	10
HEC-rosettes (mean %)	3 7	6 4
Total no of HEC rosettes counted	294	521 (χ^2 – 63 64)
Total no of mononuclear cells counted	3,966	4,061 ($P < 0$ 001)
C3 concentration in serum (mg per 100 ml, mean ±s d)	73 3±12 2	68 1±25 1

For the preparation of rosettes, 0 5 × 10^6 mononuclear cells and 0 1 ml of 2 5% complement coated red cell suspension were mixed in a final volume of HBSS of 0 5 ml and centrifuged at 200*g* for 5 min A drop of 0 02% acridin orange was added in the tube and the mixture was resuspended for 2 min on a slowly rocking mixer The counting of the rosettes was performed in a Hawksley Cristalite haemocytometer under an ultraviolet light microscope where the nucleated cells could easily be distinguished by their yellow stain All the determinations were made in duplicate To minimise the possible effect of day to day variation, an approximately equal number of F and S sera were tested in each experiment The concentration of serum C3 was determined by radial immunodiffusion[10] (M-Partigen C3c Behringwerke, Marburg, Germany)

In the first phase of the study, sera were investigated which had been frozen for 3 yr With the HEAC-technique, 10 C3F and 11 C3S sera were tested and the mean percentages of rosette-forming cells were 9 6 and 7 5%, respectively

Because of the difference it seemed worthwhile to investigate the matter further and fresh serum samples from individuals typed previously for C3 phenotypes were requested The results from this material are shown in Table 1 and Fig 1 The HEC-technique was utilised because, with the antiserum used for sensitising red cells in the HEAC technique, the background rosettes (that is, rosettes obtained with antibody coated erythrocytes) were nearly as numerous as the complement rosettes By coupling the complement on to the erythrocytes with low ionic strength the interference caused possibly by the antibodies and the respective receptors on monocytes was avoided The amount of antibody coupled on to the erythrocytes with this technique was nil or negligible, this was indicated by the fact that no rosettes were obtained

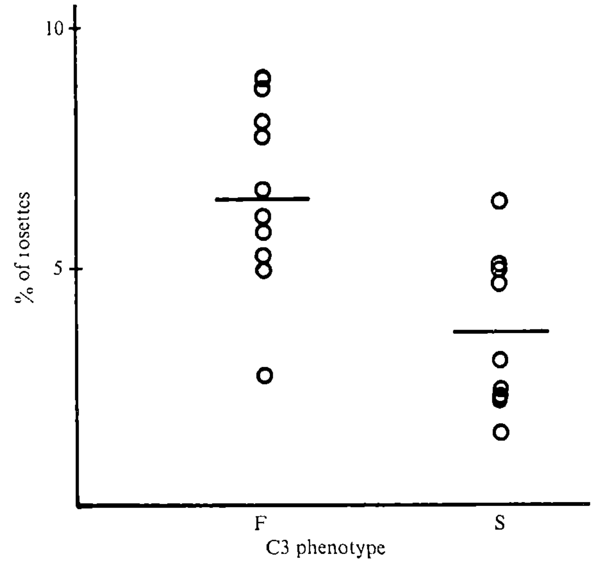

Fig 1 Influence of serum C3 phenotype on the number of HEC rosette-forming mononuclear cells Each point represents the mean of duplicate determinations The mononuclear cells used in this series of experiments originated from a single donor of C3S phenotype The difference is statistically significant ($P < 0$ 01) by Finney's first analogue of Fiellers theorem[17]

with red cells treated with fresh inactivated sera Furthermore, no immunoglobulins could be shown on the red cells with antiglobulin technique The result observed here could have arisen from differences in *in vitro* conversion between C3 F and S This is unlikely, however, as no such difference has been found previously[11,12] This possibility was further studied with fresh serum samples from two C3F and two C3S individuals The results of the experiment, completed within 5 h from the moment of venepuncture, were comparable to those in Table 1 The possibility of C3F and S being coupled on to the erythrocytes in different amounts is unlikely, too, because the red cells treated with doubling dilutions of C3F and S sera were agglutinated to similar titres with anti-complement serum It seems most probable that, compared to C3S, C3F has an enhanced capacity to bind with the respective receptor on mononuclear cells

Preliminary attempts were made to classify the cells which exhibited the receptor for complement with the present method The number of rosettes did not correlate with the number of polymorphonuclear cells in experiments with buffy coat leukocytes In two experiments where mononuclear cells were allowed to adhere on a plastic surface and were separated subsequently into adherent and nonadherent cells the HEC rosette forming cells were enriched into the former cells The mean figures were 8 4% in the initial cells suspension, 30 6% in the subpopulation of adherent cells and 1 2% in that of nonadherent cells Moreover, the majority of the cells able to form HEC-rosettes seem to possess the capacity of phagocytosing particles (unpublished results) These findings imply that the cell in question may be monocyte

In European populations the frequencies of homozygotes for $C3^S$ and $C3^F$ are roughly 60–70% and 3–5% respectively[7] My observation that C3F and C3S differ in the capacity to bind on to mononuclear cells is interesting from several points of view First, C3 seems to be one of the few genetically polymorphic human serum proteins where functional differences between the variants have been demonstrated Because a lot is known about the biological function of C3 in body defence mechanisms this-protein might deserve a closer look by geneticists, especially the supporters of the selectionist view[13]

Second, the nature of the receptor site on the membrane of the leukocytes is virtually unknown Studies with C3F and S molecules might shed more light on properties of the C3 receptor on mononuclear cells Also C3 phenotype may be another variable in studies where human complement-coated erthrocytes are used for detecting subpopulations of mononuclear cells

Thirdly, of particular interest in this connection are the findings of Bronnestam[14] and Farhud *et al*[15] They observed independently a significant surplus of $C3^F$ gene among patients with rheumatoid arthritis Furthermore, an association between $C3^S$ gene and high titres of anti-A or anti-B in newly delivered mothers[16] is another observation suggesting that C3 phenotype may have an influence on various immunological processes *in vivo* This present *in vitro* finding might offer a clue to explaining the mechanism of these phenomena

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Factors influencing microculture of leukaemia cells

THE culture of normal haemopoietic cells was accomplished[1] by obtaining colonies of granulocytic and monocytic cells from mouse bone marrow grown in a semisolid agar system The method was modified for human bone marrow and subsequent studies showed that leukaemia cells from the peripheral blood and marrow of patients with acute myelogenous leukaemia (AML) produced small colonies or clusters[1,2] Using this technique, serial sampling and morphological or biochemical studies of the growing cells was difficult A liquid culture technique was then developed to study the growth of granulocytic or monocytic cells from normal bone marrow[3] This *in vitro* diffusion chamber technique had the advantage of easy sampling but involved the use of large culture vessels The method has subsequently been used to cultivate AML cells[4]

We describe here a new short term microculture technique for growing AML cells in suspension which overcomes the cumbersome nature of the above methods and facilitates the study of multiple factors influencing the growth of these cells The method is simple, reproducible and does not require the addition of a colony stimulating factor

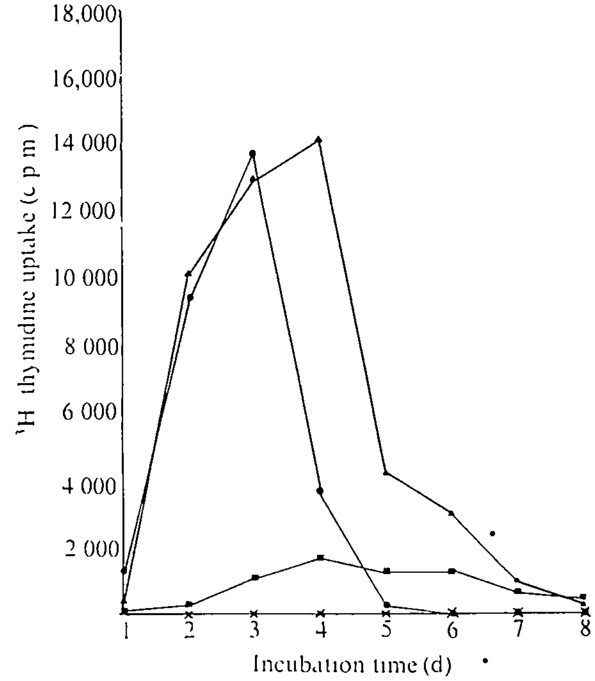

Fig 1 The optimum cell number and time of collection for the microculture of AML cells ×, 1 × 10³, ■, 1 × 10⁵, ▲, 1 × 10⁶ and ●, 1 × 10⁷ cells ml⁻¹

Both fresh and liquid nitrogen-stored leukaemia cells have been grown successfully in this system Red cells were removed from fresh heparinised blood by the addition of a 2% solution of methyl cellulose to accelerate their sedimention and the white cell layer washed once Leukaemia cells were collected from the peripheral blood of patients with AML using an NCI IBM cell separator and were stored with 10% dimethylsulphoxide in liquid nitrogen For culture, these cells were thawed rapidly, diluted slowly with RPMI 1640 medium (Biocult Limited) and then washed After washing, the cells were resuspended at a concentration of 1×10^6 ml^{-1} in medium 199 (Wellcome) containing 0 3 ml of L-asparagine, 6 6 mg ml^{-1}, and 10% foetal calf serum (Flow Laboratories) or 10% AB serum Aliquots of this suspension (0 2 ml) were added to microculture plates (Falcon 3040) Following 3 d incubation in a humid 5% CO_2 atmosphere the growth of these cells was measured by the addition of 0 1 mCi ^{3}H-thymidine (specific activity 5 Ci mmol^{-1}) to each well for 16 h The trichloracetic acid precipitable material was collected on filter paper and counted in a liquid scintillation counter

Some samples of leukaemia cells stored in liquid nitrogen showed low viability A method of slow dilution of 2 ml of original cell suspension to 20 ml over 10 min greatly improved the viability of the cells as measured by trypan blue exclusion, the incorporation of tritiated thymidine in liquid microculture and colony formation in semisolid agar. The results are shown in Table 1

A wide range of media were tested using both fresh and stored cells from several patients, and it was found that Medium 199 (Wellcome) supplemented with 10% serum and L-asparagine produced the best growth The results depicted in Fig 1 show that after an initial decline the cell concentration increased to a peak on day 4 or day 5 of culture and that DNA synthesis, as measured by the uptake of tritiated thymidine, reached a peak on day 3 or day 4 of culture This experiment also showed that the cell concentration for optimal growth in this system was 1×10^6 ml^{-1} Autoradiographs of cells obtained from these cultures showed that labelled cells had the morphological and cytochemical characteristics of AML cells This technique has therefore been shown to measure a true proliferation of AML cells in short-term culture, with an increase in cell number as well as thymidine incorporation

Table 1 Relationship between rate of dilution and viability of AML cells stored in liquid nitrogen

Rate of dilution	Initial viability	Plating efficiency (agar)	Uptake of ^{3}HTdr (liquid culture)		
Fast	64%	0 4%	6,052	6,118	
			6,165	6,656	(6,007)
			5,401	5,652	
Slow	80%	3 0%	9,913	11,728	
			10,936	12,731	(11,562)
			12,570	11,491	

More than 25 samples of acute myeloblastic and myelomonoblastic leukaemia cells have been grown from fresh blood with complete success The success rate for growing liquid nitrogen-stored cells was approximately 80% but this is now increasing as our storage techniques have improved The AML cells which multiplied in liquid culture did not mature in the normal manner After 4 d the cultures consisted almost entirely of myeloblasts and promyelocytes or primitive monocytoid cells Some small lymphocytes were also present The cultured cells from patients with acute myeloblastic leukaemia were nevertheless usually easily identified as being of the granulocytic series The typical granulation was often prominent and special stains (Sudan black and periodic acid–Schiff) were consistent with this diagnosis Cultures have also been obtained using peripheral blood cells from patients with chronic granulocytic leukaemia In these instances maturation to myelocytes and segmented forms was characteristic Leukaemia cells from the blood of ten patients with acute lymphoblastic leukaemia failed to grow using this system

This raises the possibility of using the method for diagnostic purposes in patients with acute leukaemia A relatively easy, objective test, distinguishing acute myelogenous and lymphoblastic leukaemia would be of great value Our results suggest that this method may be useful in this context A study of patients with undifferentiated leukaemia is now being made

Using the microculture technique, we have shown that sera from AML patients immunised during remission with allogenic irradiated leukaemia cells can cause growth inhibition and cell death in cultures of allogenic AML cells This property is complement dependent and is probably due to anti-HLA antibody which is known to be present in these sera using conventional assays One patient in remission with AML has been found to have complement-dependent growth inhibitory activity against autologous stored leukaemia cells In this experiment the autologous remission serum was not inhibitory in the absence of complement, but the addition of complement to the autologous serum resulted in a reduction of 80% in uptake of tritiated thymidine Complement alone did not significantly affect the uptake of thymidine Further studies to determine the frequency of this finding are under way

We feel that this new technique is of value in a number of contexts, first, as an objective, diagnostic test to distinguish acute myelogenous and lymphoblastic leukaemia, second, in studies of serum factors influencing the growth of AML cells and, third, in studies of *in vitro* sensitivity to chemotherapeutic agents

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Membrane antigens of murine leukaemia cells

THE membranes of lymphoid cells from tumours induced by oncornaviruses are antigenically altered The new surface antigen(s) are readily recognised by isologous (histocompatible) hosts which mount both cellular and humoral immune responses[1,2] It is essential to know whether or not antigens induced by the various strains of murine leukaemia viruses (MuLV) are cross-reacting immunologically

The first thorough serological study, made in a completely isologous system, was done by Old et al[3] They compared the cell membrane antigens induced by Friend virus complex (MuLV-F), Rauscher virus complex (MuLV-R), Moloney leukaemia virus (MuLV-M) and Gross virus (MuLV-G) using cytotoxic antisera from inbred mice Similarity was noted between antigens induced by Friend, Rauscher and Moloney viruses and a single cross-reacting antigenic system designated FMR was described This antigen was not found on the cells of Gross leukaemia and, conversely, antiserum prepared against Gross leukaemia had no activity for FMR This classification was readily accepted by other investigators in the field[4]

Table 1 Cell membrane immunofluorescence test with various isologous antisera*

Experiment	Antiserum†	Dilution	Leukaemia target cells (% stained) MuLV-F	MuLV-R	MuLV-M‡	MuLV-M§
A	anti-FVMA/1	1 4	>85	>85	50	
		1 8	>85	>85	<15 ‖	
		1 32	>85	60	<15	
	anti-FV/L	1 4	>85	>85	80	
		1 8	>85	>85	<15	
		1 32	50	40	<15	
	anti-MVMA/1	1 4	<15	<15	>85	>85
		1 32	<15	<15	>85	
	Normal serum	undiluted	<15	<15	<15	<15
B	anti-FVMA/2	undiluted	>90		>90	
		1 2	>90		70	
		1 4	>90		40	
		1 8	>90		<10§	
		1 32 to 1 128	>90		<10	
		1 256	50			
		1 512	<10			
anti-MVMA/2		undiluted	35		>90	
		1 2	<10		>90	
		1 4 to 1 256	<10		>90	
		1 512			80	
		1 1024			50	
		1 2048			<10	

* For each dilution, 0 035 ml of serum was added to a pellet of washed leukaemic lymphocytes (10^6) from the above preparation After incubation at 37° C for 30 min, the cells were again washed twice with buffered salt solution (Dulbecco's BSS, GIBCO) and resuspended in fluorescein-conjugated goat anti-mouse globulin (Hyland) diluted 1 15 in BSS The tubes were then incubated again at 37° C for 30 min, washed twice with BSS and resuspended in 50% glycerol-BSS for storage Cells were then examined within one week using a Zeiss microscope with fluorescent optics and a BG 12 filter For controls tested with normal mouse serum, the number of cells positive with anti-globulin in the absence of specific anti-MA did not exceed 10–15%

† Two different pools of antisera to MuLV-F cells membrane antigen (anti-FMVA/1 and 2) and of antisera to MuLV-M cells membrane antigen (anti-MVMA/1 and 2) were tested Anti-FV/L is an isologous (BALB/c) antiserum cytotoxic for Friend leukaemia cells Normal serum was pooled from BALB/c mice

‡ YAC cell line

§ Lymph node cells from leukaemic mice

‖ Percentage of cells with traces of fluorescence was as low or lower than the background (10% or 15%) with normal serum

We were, therefore, surprised to find that a battery of various isologous antisera against MuLV-F leukaemic cells prepared in our laboratory in the course of two years gave relatively little cross-reaction with MuLV-M leukaemia cells and vice versa Subsequently we learned that another investigator had a similar experience (F Lilly, unpublished) Here we report our observations suggesting similarity between MuLV-F and MuLV-R cellular antigens and their distinction from MuLV-M antigen(s) and we present a careful look at the previous data concerning the 'FMR antigen' concept

MuLV-F originally purchased from the American Type Collection (Bethesda, Maryland) was obtained from Dr W Ceglowski of Penn State University, its previous passage history on BALB/c mice was reported[5] In our laboratory, this virus was also passaged in BALB/c (ref 6) It is equally infectious for BALB/c and Swiss/ICR mice MuLV-R also passaged on BALB/c, was obtained originally from pool No 46 AP-RLV MuLV-M, described in detail elsewhere[7] was supplied by Dr M R Proffitt (Massachusetts General Hospital, Boston)

Leukaemias were induced in young adult BALB/c mice (Charles River Laboratories, Wilmington, Mass) by intraperitoneal injections of the respective virus (0 05–0 2 ml of 1% w/v, cell-free, spleen homogenate) Leukaemia cells were obtained from spleens (for MuLV-F and MuLV-R leukaemias) or from lymph nodes (for MuLV-M leukaemia) In addition, MuLV-M leukaemia cells were obtained as an established YAC cell line derived from A/Hej mice[8] and grown in our laboratory in suspension with McCoy's 5a medium and 20% foetal bovine serum

Antisera to MuLV-F-induced cell membrane antigen, FVMA, were prepared by immunisation of BALB/c mice with formaldehyde virus vaccine[9] and with several intraperitoneal injections of isologous viable leukaemic spleen cells Another anti-FVMA serum, anti-FV/L (Tables 1 and 2) was prepared in BALB/c mice by injection of live N-tropic MuLV-F followed by boosts with increasing doses of live NB-tropic virus Antiserum to MuLV-M induced cell membrane antigen (MVMA) was prepared in A/Hej mice by immunisation with the YAC cell line (derived from A/Hej mice) The first injection of irradiated cells (10^6, 6,000 rad, cobalt 60, 320 rad s^{-1}) was followed by several additional intraperitoneal injections of 10^7 live YAC cells

The specific activity of antisera was tested by indirect membrane fluorescence[10] using fresh leukaemia target cells Anti-FVMA sera reacted strongly with the membrane of MuLV-F cells and (to a lesser degree) with MuLV-R cells, 85 to 100% cells expressed the antigen (Table 1) Anti-FV/L serum (prepared in the former manner showed a similar specificity for MuLV-F and MuLV-R cells In contrast, these sera reacted very weakly, and only at high concentrations, with MuLV-M cells The cross reactivity disappeared at dilutions of 1 8 or higher The stronger of the antisera, anti-FVMA/2, had a titre of 256 with MuLV-F cells and only a titre of 4 with MuLV-M cells (Table 1, experiment B) The isologous anti-MuLV-M serum seemed to be even more specific pool 2 had a titre of 1,024 against MuLV-M cells and a titre of 2 with MuLV-F and MuLV-R membrane antigens

Cross-absorbtion studies supported the seemingly close relationship between MuLV-F and MuLV-R membrane antigens and their distinction from MuLV-M antigen (Table 2) The reactivity of anti-FVMA sera with homologous Friend cells was completely abolished by absorption with either MuLV-F or MuLV-R cells but was not diminished (at the given concentration of the antiserum) by absorbtion with MuLV-M cells Conversely, the specific activity of anti-MuLV-M serum was absorbed with MuLV-M cells but not with MuLV-F or MuLV-R cells

Table 2 Cell membrane immunofluorescence with cross-absorbed antisera to murine leukaemias*

Serum	Absorbed (cells)	Leukaemia Target Cells (% stained) MuLV-F	MuLV-R	MuLV-M
a-FVMA/1	MuLV-F	<10	<10	<10
	MuLV-R	25	<10	<10
	MuLV-M	>90	>90	<10
	Normal (control)†	>90	>90	<10
a-FV/Lilly	MuLV-F	<10	<10	<10
	MuLV-R	<10	<10	<10
	MuLV-M	85	>90	<10
	Normal (control)†	85	>90	<10
a-MVMA	MuLV-F	<10	<10	>90
±	MuLV-R	20	<10	>90
	MuLV-M	<10	<10	<10
	Normal (control)†	<10	<10	>90

*Absorption was done in three steps using equal volume of packed cells (washed thoroughly in BSS and preincubated with normal BALB/c serum for 40 min at room temperature to minimise a nonspecific loss of antibody) and undiluted antisera, 20 min at 37° C bath, 45 min at room temperature and overnight at 4° C Cells from the same source were used for both absorption and immunofluorescence assay (MuLV- M cells were YAC)

† Sera were absorbed with pooled, normal (non-infected) BALB/c spleen cells

These results argue against the concept of FMR as the major cell surface antigen common for the three leukaemias A careful evaluation of previously published work, in which the relationship between the murine leukaemia cellular antigens was studied using either serological techniques or transplantation resistance, seems to support our criticism Glynn *et al*[11] and Bianco *et al*[12] reported that BALB/c mice were protected against transplantation of Moloney lymphomas by pre-treatment with syngeneic MuLV-M cells but not with MuLV-R leukaemia cells, suggesting a lack of cross-reactivity between the transplantation antigens of the two leukaemias Using similar techniques, Glynn *et al* later reported positive finding of cross-reactivity between the F, R and M leukaemias induced in C57 Bl/6 mice[13] We found, however, that the data in their experiments showed a close relationship between F and R, but some separate specificity for M Thus, mice pretreated with MuLV-F or MuLV-R leukaemia cells were completely resistant to Friend lymphoma, whereas immunisation with MuLV-M cells conferred only a partial protection which did not seem to be much different from the protection conferred by Gross leukaemia cells[13] (Table 5)

Although it can not be assumed that the antigens responsible for transplantation immunity are necessarily the same as those involved in serological tests, the latter appears to correlate with the transplantation studies discussed above As employed by Glynn *et al*[13], membrane fluorescence showed a degree of cross-reactivity between F, M and R antigens, but there was an indication for existence of FR and M sub-groups at higher serum dilutions

The cytotoxic assays of Old *et al*[3] showed clear distinction between G and FMR antigens but results were not as clear for equally strong cross-reactivity between F, R and M because titres of antisera (particularly following *in vivo* absorbtion) were as low as 2–4 In this respect it is interesting that Klein and Klein found high titred antisera to Moloney leukaemias which reacted rather well with Gross leukaemia cells in the membrane fluorescence test but not in cytotoxic assay[10]

It seems that the number of antigen types found on the surface of leukaemia cells may be much more complex then previously thought In addition to the virus induced antigens[14,15], these might include virus envelope antigens[16,17], virus core antigens[18], foetal antigens[18,19] and other specificities[20,21] Our observations, as well as these reports, suggest that the 'FMR' term is probably misleading and it should be interpreted with caution

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Autologous human B and T lymphoblastoid cell lines

HUMAN lymphoblastoid cell lines (LCLs) have been derived from patients with acute and chronic leukaemias as well as from normal individuals[1–3] Most of these LCLs express characteristics of bone marrow-derived (B) lymphocytes[4,5] and contain the Epstein–Barr virus (EBV) genome[6] and the EBV-determined nuclear antigen (EBNA)[7] Cell lines derived from the blood of patients with myelogenous leukaemia also contain B lymphocyte markers[4,8], which may indicate that they are not of leukaemic origin In patients with lymphatic leukaemia, it has been less clear whether a derived LCL represents the neoplastic cell population Recently, LCLs derived from patients with acute lymphoblastic leukaemia were found to possess characteristics of thymus-derived (T) lymphocytes[3,5,9]

We have studied two LCLs, both derived from the same blood specimen of an 11-yr-old boy with lymphoblastic lymphoma which progressed to an acute leukaemic phase Their isolation has been previously described[10,11] We report here that these two lines displayed very different biological properties One line, CCRF-SB (here designated SB) expressed B-lymphocyte characteristics, had a human diploid karyotype, contained the EBV genome and EBNA, and activated normal allogeneic lymphocytes in the mixed lymphocyte reaction (MLR) to proliferate and develop cytotoxic capability In contrast, the other line, H-SB2, expressed T-lymphocyte characteristics, had an abnormal human karyotype, lacked detectable EBV genome and EBNA, and did not activate allogeneic lymphocytes These and other observations to be discussed suggest that the T-LCL is derived from the malignant lymphoblast population

SB and H-SB2 were examined for HL-A antigens and for the presence of five independent markers of B and T lymphocytes, as described previously[5,12] B-lymphocyte markers included surface immunoglobulin (SIg), complement C3 receptor (CR), and the human B lymphocyte-specific surface antigen (HBLA) T-lymphocyte markers included the sheep red blood cell (SRBC) receptor and the human T-lymphocyte-specific surface antigen (HTLA) EBV genome was detected by DNA–DNA reassociation kinetics analysis[13] and EBNA by anti-complement immunofluorescence[7] The capacity of the LCLs to stimulate normal peripheral lymphocytes in a one-way MLR was tested as previously reported[14] In addition, after culture for 6 d with either SB or H–SB2, these peripheral blood lymphocytes were tested for their ability to kill SB and H–SB2 in a 4 or 5 h ^{51}Cr–release assay previously described[15]

Both SB and H–SB2 grow in suspension culture and have the morphological appearance of lymphoblasts Originally, SB cells were diploid, whereas, H–SB2 cells displayed F-group trisomy in approximately 50% of the cells[16] In addition, both lines were found to share common HL–A antigens (A_1, A_2, A_{12}, W_{17}) Table 1 summarises our findings

Tests for T and B lymphocyte markers revealed that SB expressed B-cell characteristics while H–SB2 expressed T-cell characteristics Virtually all SB cells had SIg and HBLA, 15–75% formed rosettes with C3 coated SRBC, and all lacked T-cell markers In contrast, all H–SB2 cells had HTLA, 20–60% formed rosettes with unsensitized SRBC and all lacked B-cell markers

Tests for the presence of EBV (Table 2) demonstrated that all SB cells contained EBNA and that by DNA–DNA renaturation kinetics analysis the cell line contained eight genome equivalents of EBV DNA per cell H–SB2 lacked EBNA and was negative for EBV genome at the limit of the test sensitivity which was 0 2 genome equivalents per cell

The MLR tests (Table 3) revealed that SB activated the lymphocytes of three donors with stimulation indices ranging from 24 0 to 39 5, whereas, H–SB2 failed to activate lymphocytes from the same donors H–SB2 did not inhibit the response to SB when both cells were used together as stimulators, demonstrating that the absent MLR response to H–SB2 was due to a lack of stimulation capacity rather than active inhibition by H–SB2 Furthermore, SB but not H–SB2 was found to generate significant cytotoxic effector cells Lymphocytes activated by SB killed both SB and H–SB2 equally well, however, presumably on the basis of cross-reactive antigens

The heterotransplantability of SB and H–SB2 has been studied by Adams, *et al*[19] They discovered that H–SB2 could be successfully transplanted in normal neonatal Syrian hamsters, whereas SB could not be transplanted unless the recipient hamsters were first immunosuppressed with anti-lymphocyte serum In addition, H–SB2 tumours progressed to a leukaemic phase in 5% of the animals bearing tumours, whereas SB tumours never progressed to a leukaemic phase Perhaps the capacity of H–SB2 to proliferate in the untreated hamster is related to the failure of these cells to stimulate lymphocytes in the MLR

The proliferative and cytotoxic effector phases of the MLR can serve as models of immune recognition and cell-mediated immunity (CMI) *in vivo*[17] It has been suggested that the *in vitro* MLR represents the first stage of the immunological surveillance mechanism which ultimately removes abnormal lymphoid cells which arise *in vivo*[18] Therefore, the failure of H–SB2 to elicit CMI responses *in vitro* may have biological significance with respect to the *in vivo* surveillance of certain T cell malignancies

The results reported here extend previous observations[19,20] suggesting that SB and H–SB2 may be derived from different lymphoid cell populations SB had B-lymphocyte surface markers and a normal karyotype, whereas, H–SB2 had T-lymphocyte surface markers and an abnormal karyotype In addition,

Table 1 Characteristics of SB and H–SB2 cell lines

Cells	% of cells with B-lymphocyte markers*			% of cells with T-lymphocyte markers*		Presence of EBV		Stimulation of lymphocytes *in vitro*	
	SIg	CR	HBLA	SRBC	HTLA	Genome	EBNA	Proliferative response	Cytotoxic response
SB	90–100	15–75	100	0	0	+	+	+	+
H-SB2	0	0	0	20–60	100	—	—	—	—

SIg, Surface immunoglobulin, CR, complement receptor, HBLA, Human B lymphocyte antigen, SRBC, sheep red blood cell (receptor), HTLA, human T lymphocyte antigen
* Day-to-day variation occurs with some tests and is indicated by a range of values

Table 2 Presence of EBV genome and EBNA

Cells	No of EBV genome equivalents per cell*	% of cells showing EBNA†
SB	8	100
H–SB2	<0 2	0
Negative control (Calf thymus DNA)	<0 2	
Positive control (Raji cell line)	60	100

* DNA–DNA reassociation kinetics analyses were carried out as previously described[13] with 0 01 μg of ^{3}H–EBV DNA ($2\ 7\times10^6$ c p m μg^{-1}) and 2 mg of sonicated cell DNA extracted from the cultured lymphoblasts The portion of DNA reassociated was determined by hydroxyapatite column chromatography and the number of EBV genome equivalents calculated as described[13,26] EBV was purified from the extracellular fluid of the HR1K cell line

† The EBNA test was a modification of the anti-complement immunofluorescence technique described by Reedman and Klein[7] Acetone–methanol fixed cells were treated with a 1 1 mixture of human serum containing antibody against EBNA and fresh human complement obtained from an individual negative for EBV antibody (final dilution 1 10 for each) After incubation at 37° C for 45 min, the smears were washed and treated with a 1 10 diluted fluorescein isothiocyanate-conjugated anti-human β_1C/β_1A globulin at 37° C for 45 min EBNA positive cells did not react with control serum lacking antibody to EBNA

SB carried the EBV genome and EBNA, could not grow in normal neonatal hamsters, and could activate normal lymphocytes *in vitro* to proliferate and develop cytotoxic capability Similar properties have been found for other LCLs derived from normal people[6,7,19,21] In contrast, H–SB2 lacked detectable EBV genome, grew as tumours in normal newborn hamsters, and failed to activate lymphocytes to proliferate or to become sensitised killer cells Other T–LCLs, MOLT–4 (ref 3), CCRF–CEM (ref 1), and RPMI–8402 (ref 22), have displayed these same properties when examined[14,22-27] and have been derived only from patients with acute lymphoblastic leukaemia

Table 3 DNA synthesis and cytotoxic effect of lymphocytes activated by SB and H–SB2 in the mixed lymphocyte reaction

Activation mixture*	^{3}H–thymidine incorporation CPM	Stimulation index†	% ^{51}Cr release‡ ±s e from SB	H–SB2
A+SB$_m$	82,501	24 0	68 3±3 3	59 1±0 6
A+H–SB2$_m$	2,430	0 7	1 0±0 1	−17 7±0 9
B+SB$_m$	122,075	39 5	52 8±13 7	75 0±2 2
B+H–SB2$_m$	3,390	1 1	−11 8±0 4	−1 6±0 3
C+SB$_m$	108,932	31 0	91 3±3 3	84 0±6 4
C+H–SB2$_m$	2,222	0 4	0 5±0 3	13 1±0 6

DNA synthesis was measured on day 6 in a one-way MLR, as previously described[14] Each raction mixture contained $2\ 0\times10^5$ responding lymphocytes and $0\ 5\times10^5$ stimulating LCLs treated with mitomycin C The cytotoxic effect of the stimulated lymphocytes was measured on day 6 after setting up activation mixtures, as previously described[15] Cytotoxicity was measured by incubating $1\ 0\times10^6$ attacking lymphocytes with $1\ 0\times10^4$ ^{51}Cr–labelled target cells in RPMI 1640 medium containing 10% heat-inactivated foetal calf serum for 4 or 5 h at 37° C The cells were then centrifuged and the supernatant aliquots removed for counting of ^{51}Cr release Controls consisted of target cells incubated with cultured but non-sensitised lymphocytes from the appropriate donor Maximum ^{51}Cr release was obtained by freeze-thawing the labelled target cells in distilled water three times

*A, B, and C, peripheral blood lymphocytes from three donors, SB$_m$ and H–SB2$_m$, mitomycin C-treated LCLs of these lines

† Values are ratios of the mean c p m of ^{3}H–thymidine uptake in the stimulated lymphocytes and the mean c p m in the unstimulated lymphocytes (cultured alone)

‡ Values are corrected for ^{51}Cr release from control cultures

SB was probably derived, as are virtually all B–LCLs of normal people, from EBV containing normal B lymphocytes that spontaneously transform during *in vitro* culture H–SB2, on the other hand, was probably derived from the malignant T lymphoblasts of the leukaemic donor By analogy, the other known T–LCLs of leukaemic patients are probably also of neoplastic origin

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reviews

Science needed Christianity

P. E. Hodgson

Science and Creation By S L Jaki Pp vii+367 (Scottish Academic Press Edinburgh and London, July 1974) £4 50

ONE of the most intriguing questions that can be asked about the development of science is why it took place in Europe between 1250 and 1650 and not in any of the great civilisations of antiquity Ancient Babylon, Egypt and China, to take but three examples, enjoyed long periods of relative stability and developed the technological arts to a very high degree, but of science as we know it there were hardly more than a few scattered beginnings The Greeks came nearest with the mathematics of Euclid, the physics of Archimedes, the astronomy of Aristarchus and the accurate biological observations of Aristotle, but in spite of all this the enterprise never really got off the ground and soon it was all forgotten, to be rediscovered later and developed in the Middle Ages

Dr Jaki's book is a detailed examination of this problem He surveys all these civilisations as well as those of the Hindus, the Incas, the Aztecs and the Mayas to see if it is possible to isolate the reason for the many stillbirths of science before its final birth in Europe He finds that, in one way or another, all the earlier civilisations had a cyclic conception of the universe In spite of its evident absurdity this idea, with every event repeating itself exactly at regular intervals an infinite number of times, has exerted a perennial fascination on man It has always brought with it deep pessimism and despair, a sense of the futility of all effort, sapping all confidence in steady progress Why try to discover anything if it is destined all to be forgotten and then discovered again, eternally? In addition, matter was often viewed as the battleground between capricious deities, and in such an atmosphere science could not develop at all

Although we seldom recognise it, scientific research requires certain basic beliefs about the order and rationality of matter, and its accessibility to the human mind, that are seldom found in ancient civilisations They came to us in their full force through the Judaeo-Christian belief in an omnipotent God, creator and sustainer of all things In such a world view it becomes sensible to try and understand the world, and this is the fundamental reason why science developed as it did in the Middle Ages in Christian Europe, culminating in the brilliant achievements of the seventeenth century This time there was no miscarriage, and science has flourished ever since as one of the most robust movements of thought the world has ever seen

The old ideas died hard, and the history of the first 15 centuries of our era is the history of a continuous struggle between the old and the new The early Christians were not directly concerned with paving the way for science yet this was one of the results of their labours St Basil was a relentless critic of astrology, and St Augustine established the intellectual framework of an intelligible creation that enabled science to emerge centuries later The early mediaevalists, notably Adelard of Bath, insisted that a rational explanation be sought for natural phenomena and More's *Utopia* articulated the ideas that later flowered in the science of Galileo, Kepler, Copernicus and Newton

Dr Jaki develops this thesis with impressive erudition, chronicling in great detail the false starts, fantastic speculations, and occasional brilliant insights of philosophers in a wide range of cultures It is very salutary to retrace these forgotten and stumbling steps, for thereby we can appreciate more clearly the magnitude of the achievements of those who at last succeeded in hammering out the basic concepts of science as we know it, and showed how to develop and refine them by a systematic programme of reflection and experimentation ☐

Three in one

D W. Yalden

Biomechanics By Carl Gans Pp x+261 (Lippincott Philadelphia, May, 1974) Cloth $12 50, paper $5 95

BIOMECHANICS seems to have become a popular subject, and the appearance of another book on the subject might be greeted with some suspicion Were this another textbook, such suspicion might be justified, but it is nothing of the sort It is, as the author says, a highly personal, individualistic discussion of a few of the biomechanical problems on which he has worked There are just five chapters, one is introductory, the others consider in turn egg-eating snakes, legless locomotion (by snakes, amphisbaenians, and caecilians), burrowing by amphisbaenians, and anuran respiratory/vocal systems Fish, birds, and mammals get scant attention, and invertebrates none at all, yet these omissions are scarcely noticed, so interesting is the book

Each chapter commences by posing the obvious questions that a functional anatomist would ask, proceeds to find answers to them, and continues with the further questions that these answers throw up, it includes discussion of the limitations both of the answers and of the methods used to obtain them Obviously, the treatment of the topics is thorough, yet the book is by no means couched in technical language or otherwise difficult to understand The wealth of illustrations (over 150) which include explanatory graphs and complex anatomical drawings, and superb photographs, clarifies and considerably enhances the book

This is indeed a most worthwhile book on at least three levels Though not stated explicitly, it is in fact a herpetology book, and useful because of, for example, its treatment of some of the more obscure reptile groups Second, it is indeed a book of biomechanics, and has useful points to make on the application of physical principles to some quite unexpected facets of animal life Third, it is a methodology book, and I feel that for this reason in particular it deserves to be read by every zoology undergraduate who has the slightest interest in whole animal zoology Perhaps too, it ought to be read by anyone retaining the conceited view that all can be explained at the molecular level Here is the mind of a modern zoologist at work, combining time-honoured methods of comparative anatomy and new-fangled instrumentation in an attempt to understand how animals live and work

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Bony philosophy

Concepts and Approaches in Animal Morphology By P Dullemeijer Pp xii+264 (Van Gorcum Assen, Netherlands, 1974) n p

BIOLOGISTS who think about the philosophical basis of their work usually stop doing so rather quickly and scurry back to the laboratory Their unease is caused mainly by the historical and purposive aspects of biology—organisms and parts of organisms have become adapted in the past so that they perform particular functions now How can one make such systems philosophically respectable?

The main purpose of this book is to discuss the methods available to the morphologists in their attempts to determine the function of structures Dullemeijer spends much time discussing the problem of how one can make useful statements about the functions of structures, while acknowledging the truism that the functions can in no way cause the existence of the structures, as they have evolved *pari passu* The author suggests there are only three methods of much use the experimental method, the paradigm method, when crudely we think how we would do something and then see whether nature makes as good a job of it, and the comparative method, when we examine structures performing, possibly, the same function

This is a difficult book The level of abstraction is high at the beginning and end, in the middle the text becomes a detailed and somewhat controversial review of bone and bony skeletons In general this middle section would be quite interesting to someone interested in skeletons, though there are a great number of statements with which one might disagree But, if one is illustrating a principle, surely it would be better to keep to less controversial material It is too technical to be accessible to the average morphologist What is he or she to make of this, for instance "On the whole, the resistance to mechanical influence varies in the spongiosa from maximal (the trabeculae) to zero (the interspace) This at first sight self-evident, heterogeneity has an interesting consequence, because in calculations of bony structure there are specific marginal effects that have to be taken into account Such effects play a very minor effect in homogenous material Some hold the view that the elasticity modulus for spongiosa should be smaller than for compacta Considering the above-mentioned marginal effects, which will affect measurements, this seems at least doubtful"

That passage, apart from airing but not resolving a rather technical point, shows two other characteristics of this section First, most workers on bone would disagree with it the modulus of elasticity of the spongiosa is much lower than that of the compacta, that is a well attested fact Second, the quality of the English is such that one is never quite sure one has grasped the author's meaning Unfortunately, this fault is found throughout the book The phrase "specific marginal effect" for instance, is crucial to the sense of the quoted paragraph, but its meaning is quite obscure In such a philosophical book this persistent lack of clarity in exposition is most damaging to sense and, after a while, to the reader's interest

If morphologists are worried about the philosophical foundations of their work and if, unless experts, they are prepared to take the middle section on trust (which they should not), then this book may perhaps make them feel more or less comfortable It is unlikely, though, that it will have much effect on how they do their science

J D. Currey

Nucleic acids

Basic Principles in Nucleic Acid Chemistry Edited by Paul O P Ts'o Vol 1 pp xii+636, vol 2 pp xi+519 (Academic New York and London, April and June 1974) Vol 1 £18 00, $37 50, vol 2 £16 55, $34 50

AMONG multi-author books, these two volumes are unusual in that the editor has also written three long chapters which together form 37% of the text His first chapter ("In the Beginning") ranges far beyond the title of the series, from a historical review of nucleic acid research to prebiotic evolution, to contemporary biochemistry and, finally, to the destiny of man The range is so wide that it is not surprising to find occasional errors, such as the statement that cyclic AMP transmits the effects of estrogen There are also a number of spelling mistakes, and some of them also appear in the index So many facts are mentioned that a bewildered reader might be forgiven for his relief at reaching the "Concluding remarks on Evolution" (page 69) The few paragraphs in this section are, however, very confused indeed and they do not conclude the discussion of evolution "How", "why" and "fathomless" are used imprecisely, the artificial creation of living forms is irrelevantly introduced, and the difficulty of knowing the exact route of prebiotic evolution is wrongly equated with the problem of understanding gravity

In the final section of the chapter (The Beginning of the Influence of Nucleic Acid Research on the Future of Man — "Intelligent" Evolution), Ts'o argues that human nature should be altered by genetic development He states dramatically "our present genome carries the cause of our impendg destruction and has become in- lequate for the continuing evolution ' man We must develop a new species succeed *Homo sapiens sapiens*" inner's stratagem to apply behavi- ural science to avert the end of man considered to be futile ("How can controlled society be designed by be- viourists in which unknown change man is permitted and encouraged?") spice the mixture of arguments, ere are genuflexions to Teilhard de ardin and overtures to ESP Yet 'o never asks how we can hope to termine exactly which genetic anges are needed in order to alter man behaviour predictably

The other chapters are invaluable vanced reviews by authors who have ch made leading contributions to ir topics Some, such as Eisenberg d Bauer and Vinograd, have suc- eded in being critical and selective, t the general tone is encyclopaedic us, the volumes are more likely to used as a treasure house of reference than as teaching material, except very specialised postgraduate work this respect the title of the series misleading Volume 1 has a compre- sive chapter on chemical synthesis transformations of nucleosides (L odman), followed by clear, stimulat- accounts of mass spectrometry Closkey) and excited states (Gue- Eisinger and Lamola) Tsuboi's ter on infrared and Raman troscopy assumes a prior know- e of Raman spectroscopy and the volume ends with a long chapter by the editor on the structure of bases, nucleosides and nucleotides and their interactions with each other and with polymers

Volume 2 is of more general interest to biochemists It has excellent chapters on the chemical reactions of polynucleotides and nucleic acids (D M Brown), ultraviolet spectroscopy, circular dichroism and optical rotatory dispersion (Bush), hydrodynamic and thermodynamic studies (Eisenberg) and on circular DNA (Bauer and Vinograd) A final chapter by Ts'o is on the conformations and interactions of dinucleotides and oligonucleotides The two chapters by Ts'o at the end of each volume are long and very detailed indeed, but they are in a field in which he has made important and extensive contributions Naturally, there is some overlap with other chapters but it is often useful to discuss the material in a different context

Although the preface remarks on the one hundred years since Miescher, it is more to the point that nineteen years have passed since the publication of the first two volumes of *The Nucleic Acids* edited by Chargaff and Davidson Despite the presence of irrelevant material, the present volumes are particularly welcome, since the physicochemical aspects of nucleic acids have been reviewed far less than their biology and enzymology We can look forward to the later volumes of the series

K. Burton

ll about the gibbon and the siamang

bon and Siamang Vol 3 Natural 'ory, Social Behaviour, Reproduc- , Vocalization, Prehension Edit by ine M Rumbaugh Pp vii+208 rger Basel and London, 1974) 130, £18 85, $40 30, DM124

s volume contains one long paper Ellefson, reporting a field study on white-handed gibbon *Hylobates lar,* three much shorter papers These respectively, an account by kelman, Ross and Pantuwatana of ocial behaviour of a small experi- al colony of *H lar* on an island in ulf of Thailand, a description in s of functional morphology of the batid hand by R Lorenz and an ysis of vocalisations from captive ons and siamangs by Tembrock

ifortunately, much of the value ig from the fact that the papers ublished together in the one book, r than appearing separately in ials, is lost by the fact that there liaison between them For ex- le, when Brockelman *et al* and nz refer to Ellefson's work they to his unpublished doctoral dis- ion rather than to his paper in this and Tembrock's paper contains no reference to Ellefson's work at all This lack of liaison may have been no fault of the authors, but it does point to poor planning in the production of the book

The three short papers are each useful additions to the primate literature Ellefson's paper is more of a problem The blurb on the dust jacket says "Observational data have been reduced when appropriate to quantitative summaries, but their interpretation and/or possible meaning is never lost" This policy seems little short of disastrous It ensures that no reader can check the validity of Ellefson's conclusions against his data, nor does it allow detailed quantitative comparisons with published data from other primate field studies In addition to the cavalier treatment of data, the treatment of theoretical issues, such as the aetiology of vocalisations and the concept of dominance, are naive in comparison with the relatively sophisticated literature that has been published on these topics in recent years It is disappointing that what was clearly such a diligent study should have been so poorly reported

N. R. Chalmers

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214 pages 412 11040 7 £2 50

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announcements

Appointment

Desmond Gareth Julian has been appointed to the British Heart Foundation Chair of Cardiology at the **University of Newcastle-upon-Tyne**

International meetings

December 3–5, **European Conference on Irradiation Behaviour of Fuel Cladding and Core Component Materials,** Germany, (Dr D R Harries, Metallurgy Division, AERE, Harwell, Didcot, Berkshire, UK)

December 5, **The Analysis of Gaseous Effluents,** Teesside (The Society for Analytical Chemistry, Analytical Division, Chemical Society, 9/10 Savile Row, London W1X 1AF)

December 5–6, **Discussion Meeting on the Theory of Electric and Magnetic Waves in the Ionosphere and Magnetosphere,** London (The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG)

December 9–13, **Symposium of the International Atomic Energy Agency on Ionizing Radiation for the Sterilisation of Biomedical Products and Biological Tissue,** Bombay (International Atomic Energy Agency, PO Box 500, A-1011, Vienna, Austria)

December 9–14, **65th Exposition de Physique,** Paris (Societe Francaise de Physique, Comite d'exposition, 33 rue D'Exposition, 75013 Paris)

December 12, **Membrane Components Involved in Transport and Diffusion,** Oxford (Dept of Biochemistry, University of Oxford, Oxford OX1 3QU)

December 15–20, **1st Asian Regional Congress on Radiation Protection,** Bombay (Mr S D Soman, Chairman, Organising Committee RCRP, Modular Laboratories, Room 1-216H, Bhabha Atomic Research Centre, Bombay 400-085, India)

December 29, **2nd International Congress of Biorheology,** Israel (Dr F A Meyer, Weizmann Institute of Science, Rehovot, Israel)

Corrigenda

In the article "Expansion pattern of flare-associated disturbances near the Earth's orbit during October 23 to November 4, 1968" by K Sakurai (*Nature phys Sci*, **246,** 70, 1973) there were two errors in Table 1 The first number in column 1 should read 24 not 23, and the second number in the last column should read 79 5 not 69 5

In the article "Effect of foetal calf serum and epidermal growth factor on DNA synthesis in explants of chick embyro epidermis" by S Bertsch and F Marks (*Nature,* **251,** 517, 1974) the first sentence of the legend to Fig 1 should be replaced by, "Effect of epidermal growth factor (EGF) on the pulse-labelling of DNA in chick embryo epidermal explants precultivated for 24 h in the absence (*a*) or presence (*b*) of serum After precultivation, the medium was replaced by serum-free medium containing EGF and the incubation was continued for the time indicated" In the last part of the legend omit the sentence, "The explants were preincubated in MEM (without serum) for 24 h before the addition of EGF"

Erratum

In the article "Grassland species can influence the abundance of microbes on each others roots" by P Christie, E I Newman and R Campbell (*Nature,* **250,** 570, 1974) the legend to Table 2 should read, *In part *a* letters (a and b) are for comparisons among the four weights, in part *b* for comparisons within each row

Reports and Publications

Great Britain

Already Too Many (An Oxfam Special Report on Population, Family Planning and Development) By Bernard Llewellyn Pp 20 (Oxford Oxfam, 1974) [128

Bulletin of the British Museum (Natural History) Zoology, Supplement 6 The Cichlid Fishes of Lake Victoria, East Africa—The Biology and Evolution of a Species Flock By P H Greenwood Pp 1–134+1 plate (London British Museum (Natural History), 1974) £3 75 [138

Proceedings of the Royal Society of London A Mathematical and Physical Sciences, Vol 339, No 1618 A Discussion on the Exploitation of British Mineral Resources (other than Coal and Hydrocarbons) in Relation to Countryside Conservation Pp 271–416 (London The Royal Society, 1974) £2 75 [148

Bulletin of the British Museum (Natural History) Entomology Vol 31 No 1 The British Cynipoidea (Hymenoptera) Described by P Cameron By J Quinlan Pp 1–21 £1 15 Supplement 23 A Revision of the Leucospidae (Hymenoptera Chalcidoidea) of the World By Z Boucek Pp 241 £11 30 (London British Museum (Natural History), 1974) [148

The Techniques of Analytical Chemistry a Short Historical Survey By Dr H M N Irving (A Science Museum Survey) Pp 35 (London HMSO, 1974) £1 net [158

The Use of Radio Frequencies for Sound and Television Broadcasting in the United Kingdom (A paper by the BBC for its General Advisory Council) Pp 12 (London BBC, 1974) [158

Mineral Resources Consultative Committee Mineral Dossier No 8 Sulphur Compiled by A J G Northolt Pp vi + 46 (London HMSO, 1974) 47p net [158

Plant Breeding Institute Annual Report 1973 Pp 191 (Cambridge Plant Breeding Institute, 1974) £1 50 [168

National Institute of Industrial Psychology Annual Report and Statement of Accounts for the year ended September 30, 1973 Pp 61 (London National Institute of Industrial Psychology, c/o North East London Polytechnic, Livingstone Road, E15, 1974) [168

Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge—Report for 1972–73 (7th Biennial Report) Pp 126 (London Agricultural Research Council, 1974 Obtainable from HMSO) £1 30 net [168

Select Committee on Science and Technolo Government Observations on Third Report, Sessi 1972–73—Tracked Hovercraft, Ltd Pp 11 (Cm 5711) (London HMSO, 1974) 11p net [1

British Aid and the Relief of Malnutrition—Rep of the ODA Advisory Committee on Protein Pp (London Ministry of Overseas Development, 1974 [1

Bulletin of the British Museum (Natural Histo Zoology, Vol 27, No 2 Miscellanea Pp 49–138 plates (London British Museum (Natural Histo 1974) £4 70 [

National Radiological Protection Board NRPB R The Study of Chromosome Aberration Yield in Hum Lymphocytes as an Indicator of Radiation Dose A Review of Cases Investigated—1973 By R Purrott, D C Lloyd, J S Prosser, G W Dolp Elaine J Eltham, Patricia A Tipper, Carolyn M W and Susan J Cooper Pp 27 (Harwell, Didc National Radiological Protection Board, 1974) [

Journal of Biogeography, Vol 1, No 1, March 19 Edited by David Watts Pp 1–74 Published Quarte Annual subscription £10 (USA and Canada $ (Oxford Blackwell Scientific Publications, Ltd , 19 [

British Antarctic Survey Scientific Reports, No The Dynamics of the Brunt Ice Shelf, Coats La Antarctica By R H Thomas Pp 45 + 3 pla (London British Antarctic Survey, Natural Envir ment Research Council, 1973) £2 75 net [

Other Countries

CERN—European Organisation for Nuclear search Annual Report for 1973 Pp 235 (Gen CERN, 1974)

United States Department of the Interior Geolo Survey Professional Paper 804 Erosional and De tional Aspects of Hurricane Camille in Virginia, By Garnett P Williams and Harold P Guy Pp 80 (Washington, DC Government Printing O 1973) $1 80

International Directory of Woody Plant Physiolo Compiled by Gregory N Brown and James R Bra (Columbia, Missouri Forest Service, US Depart of Agriculture, in co-operation with School of Fore Fisheries and Wildlife, University of Missouri, 197

International Atomic Energy Agency Annual R July 1, 1973–June 30, 1974 Pp 75 The Agency' gramme for 1975–80 and Budget for 1975 (Vi International Atomic Energy Agency, 1974)

Fondation Universitaire, Bruxelles 53e Ra Annuel Pp 95 (Bruxelles Fondation Univers 1974)

World Health Organization Technical Report S No 552 WHO Expert Committee on Tubercul Ninth Report Pp 40 (Geneva WHO, Lo HMSO, 1974) Sw fr 5

FAO/WHO/OIE Animal Health Yearbook Pp 199 (Rome FAO, London HMSO, 1974) £ $4 50

Publications du Centre National pour l'Exploit des Oceans (CNEXO) Fascicule 2, 1974 Receu Travaux du Centre Oceanologique de Bretagne (Pp 652 (Paris, Cedex CNEXO 1974)

Organisation for Economic Co-operation Development Mercury and the Environment St of Mercury Use Emission, Biological Impact Control Pp 196 (Paris OECD, London H 1974) 25 francs, £2 50, $6 25

United States Department of the Interior Geo Survey Water-Supply Paper 2027 Analog Model of the Ground-Water Basin of the Upper Coa Valley, California By Stephen J Tyley Pp v $1 05 Professional Paper 387–B Recent Activ Glaciers of Mount Rainier, Washington By Rob Sigafoos and E L Hendricks Pp vi + 24 (Washington, DC Government Printing Office and 1974)

Contribucions del Instituto Antartico Argentir 107 Segunda Contribucion al Conocimien Microplancton del Mar de Bellingshausen Por Balech Pp 63 No 142L Distribucion Vertic Fauna Benromica en Tres Localidades Anta Bahia Esperanza, Isla Petermann y Arch Melchior Por N B Bellisio, R B Lopez y Tomo Pp 87 No 152 Interpretacion de las ciones Aurorales en Terminos de Fenomenos O torios Por H A Cazeneuve Pp 19 No 155 E Diatomologico del Mar de la Flota, de Puerto P y Observaciones en el Mar de Bellingshause L J C Martinez Macchiavello Pp 103 No Biosintesis de Esteroides por la Glandula Supra de la Foca de Weddell—un Ejemplo de Ada Evolutiva y Ecologica Por M A Borruel, P B M C Damasco y C P Lantos No 161 Notas gicas sobre la Isla Pedro I Por L A P Tomo No 162 Geologia del Sector Noroccidental Peninsula Hurd, Isla Livingston, Shetland d Antartica Argentina Por R Caminos, *et al* No 166 Estudio *in vitro* sobre el Mecanis Secrecion de Insulina en el Pinguino Papua (Py Papua) Por J M S Farina y R A Chieri (Buenos Aires Direccion Nacional del An Instituto Antartico Argentino, 1972 y 1973)

New Zealand National Roads Board RRU No 17 Vehicle Occupancy, Trip Purpose, Traff position and Operating Speed—Feasibility Stu Pilot Survey Pp 48 (Wellington, NZ Nationa Board, Road Research Unit, 1973)